CANADIAN JOURNAL OF RESEARCH

VOLUME 15 NOVEMBER, 1937 NUMBER 11

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NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 15, SEC. C. NOVEMBER, 1937 NUMBER 11

FUSARIUM SPP. AS ROOT PARASITES OF ALFALFA AND SWEET CLOVER IN ALBERTA[1]

BY M. W. CORMACK[2]

Abstract

Five pathogenic species predominated among the numerous isolates of *Fusarium* obtained from diseased roots of alfalfa and sweet clover in Alberta. Of these, the closely related species *F. avenaceum* (Fr.) Sacc. and *F. arthrosporioides* Sherb. appear most important, because they occur commonly and can cause serious injury to the roots, both in the early spring and during the growing season. *F. culmorum* (W.G.Sm.) Sacc. is very virulent during the summer, but is apparently non-pathogenic in the early spring. At both times *F. Poae* (Peck) Wr. and *F. Scirpi* Lamb. et Fautr. var. *acuminatum* (Ell. et Ev.) Wr. usually behave as weak pathogens. With the exception of *F. avenaceum* on alfalfa and sweet clover, and *F. Scirpi* var. *acuminatum* on alfalfa, these species have not been previously reported as occurring on the host plants indicated.

Cardinal temperatures for growth in pure culture were:— *F. avenaceum* and *F. arthrosporioides:* −2°, 24°, and 34° C.; *F. culmorum*: 3°, 24° to 27°, and 34° to 36° C.; *F. Poae*: −2°, 20° to 24°, and 32° C.; *F. Scirpi* var. *acuminatum:* 1°, 24°, and 34° C. All five species grew well at hydrogen ion concentrations ranging from pH 4.0 to 9.5. Carbon dioxide concentrations up to 20% had very little effect on the growth of *F. avenaceum*, *F. arthrosporioides*, or *F. Poae*, but the higher concentrations retarded the growth of *F. culmorum* and *F. Scirpi* var. *acuminatum*. The retarding effect of carbon dioxide was greater at 5° C. than at room temperature.

F. avenaceum produced more infection at temperatures up to 24° C. than at 27° C. At 27° C., infection was much lighter in dry soil than in moist soil. *F. culmorum* caused severe damage at 18° to 27° C., but did not attack the roots at low temperatures. *F. avenaceum* usually attacked roots of sweet clover more severely than those of alfalfa. All varieties of both hosts tested proved susceptible. In the absence of wounds, *F. avenaceum* readily entered roots through the basal tissues of branch roots, or through lenticels. Variant forms of this pathogen, which occurred frequently in pure culture, proved decidedly less pathogenic than the original isolates.

Alfalfa and sweet clover roots were attacked by an isolate of *F. avenaceum* obtained from diseased roots of *Vicia americana*. *F. avenaceum*, *F. arthrosporioides*, and *F. culmorum* from alfalfa and sweet clover proved pathogenic to roots of *Trifolium* spp. and to seedlings of wheat, oats, and barley. Certain isolates from the cereals were pathogenic to roots of alfalfa and sweet clover, and thus certain limits to crop rotation in reducing the root-rot damage caused by these pathogens are indicated.

During recent years several fungi have been found parasitizing roots of alfalfa and sweet clover in Alberta. *Plenodomus meliloti* Dearness and Sanford (12) and *Cylindrocarpon Ehrenbergi* (5) have proved of primary importance following the winter dormancy period, while *Sclerotinia* sp. (4) has been

[1] *Manuscript received July 24, 1937.*

Contribution No. 512 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada, co-operating with the Department of Field Crops, University of Alberta. This paper constitutes one part of a thesis presented to the Faculty of the Graduate School of the University of Minnesota, in partial fulfilment of the requirements for the degree of Doctor of Philosophy, December, 1936.

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destructive during the growing season, as well as in the early spring. At both times *Fusarium* spp. predominated among the other fungi isolated from diseased roots, and the prevalence of certain species suggested a parasitic relationship. After eliminating the purely saprophytic forms in preliminary pathogenicity tests, a detailed study was made of the pathogenic isolates representing the following species: *F. avenaceum* (Fr.) Sacc., *F. arthrosporioides*, Sherb., *F. culmorum* (W. G. Sm.) Sacc., *F. Poae* (Pk.) Wr., and *F. Scirpi* Lamb. et Fautr. var. *acuminatum* (Ell. et Ev.) Wr. This investigation has been chiefly concerned with the root rots of alfalfa and sweet clover caused by these species, and with the host relations and physiology of the isolates.

Review of Literature

In a recent book by Wollenweber and Reinking (21), 20 distinct species or varieties of *Fusarium* are listed as occurring on alfalfa, and three species are listed for sweet clover. These include *F. avenaceum* on alfalfa and sweet clover, and *F. Scirpi* var. *acuminatum* on alfalfa, but no detailed studies have been reported on root rots caused by these species. The other three species studied herein, namely *F. arthrosporioides*, *F. culmorum*, and *F. Poae* have apparently not been previously reported on alfalfa or sweet clover. However, all five species are widely distributed, and are important parasites of other plants (20).

Root diseases of alfalfa and sweet clover caused by *Fusarium* spp. have received very little detailed attention in the past. The most comprehensive study reported is that of Weimer (17), on the wilt disease of alfalfa in Mississippi caused by *F. oxysporum* var. *Medicaginis*. Other reports are mainly observational and frequently do not include proof of parasitism or identification of the species concerned. Since most of these reports are reviewed by Weimer (17), only those having a bearing on the present problem will be mentioned here.

Fergus and Valleau (7) found that more than 15 distinct species of *Fusarium*, obtained from alfalfa roots and other sources, were highly pathogenic to sterile alfalfa and clover seedlings growing on agar in test tubes. Other workers, using more natural conditions in their infection experiments, have obtained quite different results. Weimer (18) found that *Fusarium* spp. predominated in the tissues of alfalfa roots suffering from winter injury, but concluded, from the results of infection experiments, that these fungi were mainly saprophytes, or weak parasites. Peltier and Tysdal (11) came to a similar conclusion regarding several species of *Fusarium* which they isolated from rotted alfalfa roots. For many years alfalfa root rot attributed to *Fusarium* spp. has been reported from Missouri, but Scott (14) finally decided that invasion of the roots by these fungi under natural conditions was largely secondary, and occurred in wounds caused by cultural practices or winter injury. As far as is known, none of the above-mentioned studies were concerned with the species found most pathogenic in the present investigation.

Prevalence in Alberta

Under natural conditions, root rot of alfalfa and sweet clover caused by *Fusarium* spp. cannot be distinguished with certainty from that produced by other pathogens. However, isolation studies give some indication of the relative prevalence of the pathogenic species.

All five species of *Fusarium* studied were isolated from diseased roots collected at widely separated points in the principal soil zones of Alberta, although none of them were as prevalent as *Cylindrocarpon Ehrenbergi* (5). The three most pathogenic species, namely *F. avenaceum*, *F. arthrosporioides*, and *F. culmorum*, occurred most frequently. Also, each of these species was almost invariably the only pathogen obtained from the diseased roots from which it was isolated. *F. avenaceum* occurred with about equal frequency on alfalfa and sweet clover, and was isolated from about 20% of the diseased root samples taken from 45 different fields. This species was also isolated from the soil of several alfalfa fields and from diseased roots of *Vicia americana*. The closely related species, *F. arthrosporioides*, occurred on about 10% of the roots examined. *F. culmorum* occurred only on the roots of plants which were damaged during the summer. The most pathogenic isolates of this species were obtained from sweet clover roots in southern Alberta. *F. Poae* and *F. Scirpi* var. *acuminatum* occurred frequently on diseased roots of both alfalfa and sweet clover, but usually in association with a more virulent pathogen, such as *F. avenaceum*.

Infection Studies

Infection of Roots by *Fusarium* spp. Compared

Representative isolates of the species which proved pathogenic in preliminary tests were studied more thoroughly in several winter and summer field experiments. Roots of Grimm alfalfa and Arctic sweet clover were inoculated and the degree of infection was estimated, as recently described (5). The results given in Tables I and II show that *F. avenaceum* was one of the most pathogenic species in both winter and summer tests. It caused a typical root rot, which was particularly severe following the winter dormancy period (Table I). Infection was first observed shortly after thawing occurred in the soil, and it was well advanced when growth started. At that time the brownish lesion formed on each inoculated root was slightly sunken toward the centre, and usually had a narrow, dark brown, or nearly black margin (Plate I, A). Lesions formed during the summer were usually less clearly defined (Plate I, C). Subsequent progress by *F. avenaceum* sometimes resulted in the rotting of the entire root system, or in the formation of large rotted areas which greatly weakened or eventually killed the plant. Sweet clover roots were generally more severely attacked than those of alfalfa. All isolates studied were pathogenic, including one obtained from a diseased root of *Vicia americana*.

F. arthrosporioides, which is closely related taxonomically to *F. avenaceum* (20), was the only other species studied which proved highly pathogenic in

TABLE I

RELATIVE PATHOGENICITY OF SPECIES OF *Fusarium* ON ROOTS OF ALFALFA AND SWEET CLOVER IN THE EARLY SPRING

(Winter tests, 1935–36 and 1936–37)

Species	Isolate		Infection rating*, %					
			Alfalfa			Sweet clover		
	No.	Source	1935–36	1936–37	Ave.	1935–36	1936–37	Ave.
F. avenaceum	1	Sw. clover	28	32	30	50	84	67
	6	Alfalfa	38	44	41	55	84	69
	9	Sw. clover	42	25	33	47	60	53
	14	Alfalfa	37	31	34	50	59	54
	39	Vetch		45			87	
F. arthrosporioides	7	Alfalfa	22	40	31	32	77	54
	35	Alfalfa		58			81	
	38	Sw. clover		50			81	
F. culmorum	2	Sw. clover	7	1	4	0	2	1
	37	Alfalfa		5			3	
F. Poae	3	Alfalfa	13	10	11	23	13	18
	4	Alfalfa	4	6	5	15	5	10
	13	Sw. clover		17			4	
F. Scirpi var. *acuminatum*	8	Alfalfa	5	12	8	2	15	8
	17	Alfalfa		8			5	
	34	Sw. clover		2			10	
Check plants			6	1	3	0	0	0

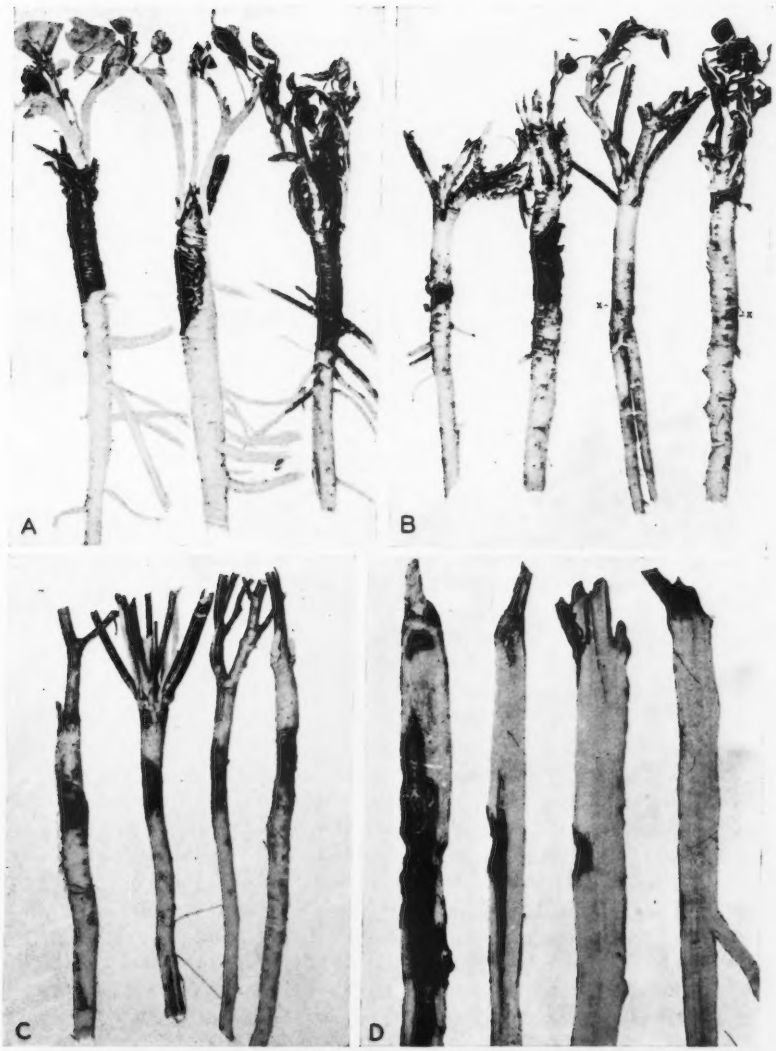
* *Average numerical rating of 15 plants in each test.*

the early spring, as well as during the growing season (Tables I and II). It produced symptoms on the roots which were indistinguishable from those caused by *F. avenaceum* (Plate I, B).

F. culmorum did not attack roots of alfalfa and sweet clover in the early spring (Table I), but during summer it was the most pathogenic species studied (Table II). Invasion of the roots by this species was particularly rapid in July and August, when sweet clover plants were often dead within two weeks after the roots were inoculated. Wilt symptoms, resembling those described by Weimer (17) for *F. oxysporum* var. *Medicaginis*, were often produced by the sudden dying of plants during summer, but examination of the roots usually revealed a typical root rot. The root lesions resembled those produced by *F. avenaceum*, but they were usually darker in color and more extensive (Plate I, C).

F. Poae and *F. Scirpi* var. *acuminatum* proved weakly pathogenic on roots of alfalfa and sweet clover in both winter and summer tests (Tables I and II). Some isolates appeared slightly more pathogenic than others and caused light to moderate infection during the summer. These species produced small, brown, and usually superficial lesions on inoculated roots (Plate I, B).

PLATE I



Roots of alfalfa and sweet clover artificially inoculated with Fusarium spp. Fig. A. Early spring infection by F. avenaceum. Left, two plants of Arctic sweet clover; right, Grimm alfalfa. Fig. B. Early spring infection by F. arthrosporioides, alfalfa and sweet clover on left, and by F. Poae, alfalfa and sweet clover on right. X indicates invaded areas in the latter case. Fig. C. Summer infection by F. avenaceum, sweet clover and alfalfa on left, and by F. culmorum, alfalfa and sweet clover on right. Fig. D. Influence of soil temperature and soil moisture on infection of sweet clover roots by F. avenaceum. Left to right: 24° C. and 60% M.H.C.; 27° C. and 60% M.H.C.; 27° C. and 40% M.H.C.; control, 24° C. and 60% M.H.C.

TABLE II

RELATIVE PATHOGENICITY OF SPECIES OF *Fusarium* ON ROOTS OF GROWING PLANTS OF ALFALFA AND SWEET CLOVER

(Summer tests, 1935 and 1936)

Species	Isolate		Infection rating*, %							
			Alfalfa				Sweet clover			
	No.	Source	Test			Ave.	Test			Ave.
			1	2	3		1	3	3	
F. avenaceum	1	Sw. clover	26	35	21	27	54	51	47	51
	6	Alfalfa	24	17	17	19	34	29	29	31
	9	Sw. clover	22	31	17	23	26	34	34	31
	14	Alfalfa	22	25	17	21	32	23	27	27
	39	Vetch			25				35	
F. arthrosporioides	7	Alfalfa	17	13	11	14	36	44	42	41
	38	Sw. clover			26				52	
F. culmorum	2	Sw. clover	42	42	43	42	60	70	71	67
	37	Alfalfa			39				50	
F. Poae	3	Alfalfa	18	15	14	16	20	26	23	23
	4	Alfalfa	16	11	8	12	18	13	14	15
	13	Sw. clover		18	13			31	22	
F. Scirpi var. *acuminatum*	8	Alfalfa	22	21	15	19	29	32	36	32
	17	Alfalfa		18	16			29	30	
	34	Sw. clover			11				23	
Check plants			8	5	4	6	10	8	5	9

* *Average numerical rating of 15 plants in each test.*

PATHOGENICITY OF VARIANT TYPES

The original isolates of *F. avenaceum* from diseased roots were of the typical mycelial type, since they produced long, abundant, fluffy, white to pink mycelium on potato-dextrose agar. Subsequently, three variant types developed so frequently from this long mycelial form that they seemed to warrant detailed study. One type, occurring commonly as sectors or patches in plate culture, produced short, felted, reddish mycelium. This short mycelial type often produced sectors and patches of the original type when cultured. Cultures of the pionnotal type described by Brown (3) occurred occasionally as sectors in plate cultures of the original isolate, and were readily obtained by transferring single spores from old cultures. All attempts to make this heavily sporulating type revert to the mycelial form have so far failed. A semi-pionnotal type, with scant, matted, white mycelium intermingled with orange masses of spores, was also sometimes obtained by transfer of single spores. These variant types are of great interest from a genetical and cytological standpoint. They are being studied further, but only their relative pathogenicity will be reported at this time.

In infection experiments with alfalfa and sweet clover, the three variant types proved decidedly less pathogenic than the original isolate (Table III). Two cultures of the short mycelial type were only slightly pathogenic during

TABLE III

PATHOGENICITY OF VARIANT FORMS OF *Fusarium avenaceum* ON ROOTS OF ALFALFA AND SWEET CLOVER AS COMPARED WITH THAT OF THE ORIGINAL ISOLATE

Culture		Infection rating*, %							
		Alfalfa				Sweet clover			
Type	No.	Winter test	Summer tests			Winter test	Summer tests		
			1	2	Ave.		1	2	Ave.
Long mycelial (original)	1	32	27	23	25	84	68	44	56
Short mycelial	1b	8	11	5	8	40	21	11	16
Short mycelial	1c	8	14	11	12	47	32	13	22
Pionnotal	1d	7	19	18	18	14	49	21	35
Pionnotal	1e		14	11	12		37	14	25
Semi-pionnotal	1f	4	8	3	5	5	15	5	10
Check plants		1	0	0	0	0	12	0	6

* *Average numerical rating of 15 plants in each test.*

summer, but they caused moderate infection of sweet clover roots in the early spring. When re-isolations were made from the infected roots, the original long mycelial type was often obtained. The pionnotal cultures were usually somewhat more virulent than those of the short mycelial type, but the semi-pionnotal culture never caused more than a trace of infection. The pionnotal type was usually recovered from the infected root tissues. Other workers have also found that pionnotal cultures had reduced virulence. In studies of *F. avenaceum* and *F. culmorum* from carnations, Wickens (19) found that pionnotal strains were non-pathogenic, although they were derived from virulent mycelial strains.

INFLUENCE OF SOIL TEMPERATURE ON INFECTION

Under field conditions, the amount of infection produced by *F. culmorum* on roots of alfalfa and sweet clover appears to be correlated with the soil temperature. For example, two isolates were highly pathogenic in July, when the soil temperature averaged 20° C. for the period of the experiment, but they produced only light infection in a September experiment, when the soil temperature averaged 12° C. On the other hand, isolates of *F. avenaceum* and *F. arthrosporioides* caused approximately the same degree of infection in September as they did in July. These results were confirmed in a greenhouse experiment in which roots of dormant alfalfa and sweet clover plants, taken from frozen soil, were inoculated and transplanted into boxes of soil held at average temperatures of 1.5°, 12°, 17°, and 21° C. *F. avenaceum* produced moderate to heavy infection at all four temperatures, but *F. culmorum*

attacked the plants only at 17° and 21° C. This relation of temperature to infection probably explains why *F. avenaceum* can cause severe damage to the roots in the early spring, while *F. culmorum* is non-pathogenic at that time.

The influence of relatively high soil temperatures on infection of sweet clover roots by *Fusarium* spp. was studied during the summer in soil temperature control tanks. The roots of field-grown plants were inoculated and transplanted into pots containing three parts of black soil mixed with one part of sand, which were kept at temperatures of 18°, 21°, 24°, and 27° C. In the first experiment, where the soil moisture was maintained at approximately 60% of the moisture holding capacity of the soil (M.H.C.), *F. culmorum* produced nearly maximum infection at all temperatures studied (Table IV),

TABLE IV

INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE ON INFECTION OF SWEET CLOVER ROOTS BY *Fusarium avenaceum* AND *F. culmorum*

Pathogen	Expt.	Infection rating*, %							
		40% M.H.C.†				60% M.H.C.†			
		18°	21°	24°	27°‡	18°	21°	24°	27°‡
F. avenaceum	1					80	75	82	58
F. avenaceum	2	76	70	82	27	72	68	87	52
F. culmorum	1					97	96	94	94
Check plants	1					5	0	3	5
Check plants	2	0	0	0	1	0	0	0	1

* *Average numerical rating of 30 plants.*
† *Soil moisture expressed as percentage of moisture holding capacity.*
‡ *Soil temperature in degrees Centigrade.*

but *F. avenaceum* attacked the roots less severely at 27° C. than at the lower temperatures. In a second experiment, using only *F. avenaceum*, the soil moisture in duplicate sets of pots, held at each temperature, was kept at 40 and 60% M.H.C., respectively. The roots were severely rotted at 18°, 21°, and 24° C., in both dry and moist soil (Table IV). At 27° C. injury was most markedly reduced in the relatively dry soil, where only light infection occurred (Plate I, D). The indicated differential effect of soil moisture was confirmed in another experiment carried out at greenhouse temperature, where the roots were severely attacked by *F. avenaceum* in wet soil (70% M.H.C.), but were only moderately injured in soils of 55 and 35% M.H.C.

VARIETAL AND HOST RANGE TESTS

A study of varietal reaction to *F. avenaceum* has been started, since this species appears to be the most prevalent of those studied. Several commonly grown varieties of alfalfa and sweet clover, representing different species of *Medicago* and *Melilotus*, have been tested by direct inoculation of the roots. The results obtained in two winter tests and one summer test (Table V) indicate that all the species and varieties so far tested are more or less sus-

TABLE V

REACTION OF VARIETIES OF *Medicago* AND *Melilotus* AND SPECIES OF *Trifolium* TO ROOT ATTACK BY *Fusarium avenaceum*

Species and variety	Infection rating*, %			
	Winter tests			Summer tests, 1936
	1935–36	1936–37	Average	
Medicago falcata	12	20	16	16
Medicago sativa				
Hardistan	16	52	34	9
Medicago media				
Cossack	14	27	20	10
Grimm	12	46	29	13
Ladak	9	59	34	13
Ontario Variegated	13	62	37	10
Melilotus alba				
Arctic	27	81	54	43
Alpha No. 1	46	94	70	54
Brandon Dwarf	31	85	58	49
Grundy County	43	82	62	43
White Blossom	25	71	48	30
Melilotus officinalis				
Albotrea	45	90	67	48
Yellow Blossom	28	82	55	43
Zouave	29	78	53	53
Trifolium hybridum	13			24
T. pratense	23			24
T. repens	23			28

* *Average numerical rating of 40 plants of each variety in each test.*

ceptible to attack by *F. avenaceum*. None of the species showed the consistent resistance displayed by *Medicago falcata* and *Melilotus officinalis* to attack by *Sclerotinia* sp. (13). However, all varieties of sweet clover were more severely attacked than those of alfalfa. The widely grown White Blossom and Arctic varieties had a slight but consistently lower infection rating than the other sweet clover varieties tested. Further tests are necessary before definite conclusions can be drawn.

F. avenaceum produced light to moderate infection on roots of *Trifolium hybridum*, *T. pratense*, and *T. repens* in winter and summer tests (Table V). In the summer *F. culmorum* was also pathogenic on roots of *Trifolium* spp., and usually caused more damage than *F. avenaceum*.

CROSS INOCULATION STUDIES

All five species of *Fusarium* that proved pathogenic on roots of alfalfa and sweet clover in this study also occur on cereal crops (8). In fact, *F. avenaceum* and *F. culmorum* are best known as the cause of root rot and blight

of wheat, oats, and barley (1, 15). The possible existence of host specialization in these species seemed to warrant at least a preliminary study, so cross inoculations were made on the cereals and legumes. In these experiments, isolates of *F. avenaceum* from wheat, oats, and barley, supplied by Dr. W. L. Gordon of the Dominion Rust Research Laboratory, Winnipeg, and two cultures of *F. culmorum*, isolated from wheat roots by Dr. W. C. Broadfoot of this laboratory, were tested in comparison with some of the isolates obtained from roots of alfalfa and sweet clover.

In field infection experiments, the isolates of *F. avenaceum* from oats and barley caused moderate and light infection, respectively, on roots of sweet clover, but they were only slightly pathogenic to alfalfa (Table VI). The

TABLE VI

RELATIVE PATHOGENICITY OF ISOLATES OF *Fusarium avenaceum* AND *F. culmorum* FROM LEGUMES AND CEREALS ON ROOTS OF ALFALFA AND SWEET CLOVER

Pathogen	Isolate		Infection rating*, %							
			Alfalfa				Sweet clover			
	No.	Source	Winter tests	Summer tests			Winter test	Summer tests		
				1	2	Ave.		1	2	Ave.
F. avenaceum	1	Sw. clover	32	27	23	25	84	68	44	56
	14	Alfalfa	31	39	25	32	59	49	32	40
	1070	Barley	4	11	5	8	33	28	8	18
	1092	Oats	10	8	8	8	68	52	27	39
	1203	Wheat	2	6	1	3	1	11	4	7
F. culmorum	2	Sw. clover	1	43	42	42	2	71	69	70
	B35	Wheat	3	30	41	35	3	68	51	59
	B155	Wheat	2	24	12	18	3	70	32	51
Check plants			1	8	0	4	0	12	0	6

* *Average numerical rating of 15 plants in each test.*

isolate from wheat was non-pathogenic, while those from alfalfa and sweet clover were moderately to highly virulent, as previously indicated. On the other hand, the isolates of *F. culmorum* from wheat were nearly as virulent as an isolate obtained from sweet clover, since they produced medium infection of alfalfa roots, and medium to heavy infection of sweet clover roots in summer tests (Table VI). In a winter test, all three isolates of *F. culmorum* were non-pathogenic.

The isolates of *F. avenaceum* and *F. culmorum* discussed above, and also one isolate of *F. arthrosporioides* from alfalfa, were tested in the greenhouse for pathogenicity on seedlings of barley, oats, and wheat. Twenty surface-disinfected seeds were planted immediately above ten grams of oat-hull inoculum in each pot of sterilized soil. Emergence notes were taken in ten days, and the experiments were concluded at the end of 30 days. At that time, the degree of infection of each root was estimated numerically, using the values

from 0 to 10, and maximum rating was given to all plants which died after emergence. The results of one experiment, summarized in Table VII, show that the isolates of *F. avenaceum* from alfalfa and sweet clover were more

TABLE VII

RELATIVE PATHOGENICITY OF ISOLATES OF *Fusarium* SPP. FROM LEGUMES AND CEREALS TO SEEDLINGS OF BARLEY, OATS, AND WHEAT

Pathogen	Isolate		Germination, %			Infection rating*, %		
	No.	Source	Barley	Oats	Wheat	Barley	Oats	Wheat
F. avenaceum	1	Sw. clover	74	97	72	75	31	74
	14	Alfalfa	95	95	38	49	25	88
	1070	Barley	93	93	48	41	8	65
	1092	Oats	95	100	87	35	6	31
	1203	Wheat	97	100	93	4	1	10
F. culmorum	2	Sw. clover	85	63	87	57	70	72
	B35	Wheat	93	76	90	57	56	62
	B155	Wheat	91	58	46	43	51	72
F. arthrosporioides	7	Alfalfa	97	92	80	28	31	72
Check plants			97	98	93	1	3	3

* *Average numerical rating of plants in four pots.*

pathogenic than those from the cereals, since they caused medium to heavy infection of barley and wheat, and light to medium infection of oats. The isolate from barley was, however, moderately pathogenic to wheat and barley, but none of the isolates from cereals caused appreciable damage to oats. Isolate 1203 from wheat was non-pathogenic on the cereals, as well as on alfalfa and sweet clover (Table VI). This culture was of the pionnotal type previously discussed, which may explain its non-virulence. The three isolates of *F. culmorum* studied were approximately equal in virulence, regardless of their origin, and produced medium to heavy infection of wheat, oats, and barley. *F. arthrosporioides* isolated from alfalfa proved highly pathogenic to wheat, and caused light to medium infection of oats and barley.

Pathological Anatomy

Since preliminary studies indicated that similar histological features were concerned in the invasion of roots by *F. culmorum* and by *F. avenaceum*, only the latter was chosen for more detailed investigation. Material showing the progressive stages of infection was obtained by inoculating non-wounded roots of alfalfa and sweet clover in the field. Sets of five roots, taken up at daily intervals after inoculation, were prepared for histological examination by the methods previously described (5).

The absence of wounds did not appear to retard penetration of the roots by *F. avenaceum*. Within two days after inoculation, hyphae were frequently observed in the basal tissues of branch roots, and in the loose outer tissues

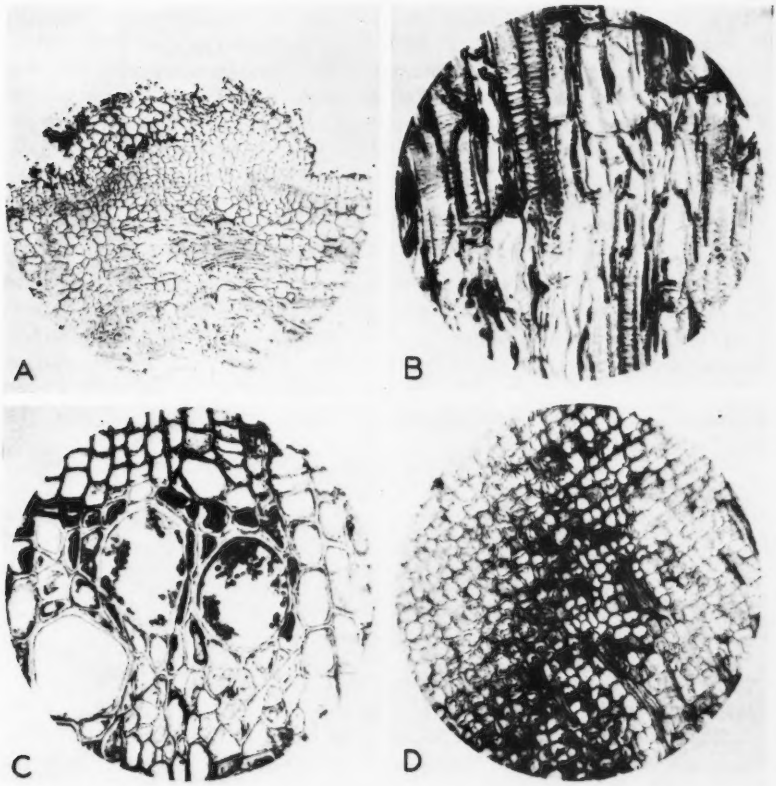


FIG. 1. *Invasion of roots of Fusarium avenaceum. A. Hyphae starting to enter an alfalfa root through a lenticel, two days after inoculation. ×85. B. Hyphae proceeding into a tap root of sweet clover by way of the connective vascular tissue of a branch root. ×330. C. Cross-section of a portion of the vascular region of a tap root showing the cut ends of hyphae which are progressing longitudinally in the vessels. ×330. D. Line of demarcation at border of an old lesion. Uninvaded area at right. ×130.*

of lenticels (Fig. 1, A). The thin cork layers below these points usually offered but little resistance to the pathogen. When infection occurred at the base of a branch root, the hyphae often passed into the tap root by way of the connective vascular tissues of the branch root (Fig. 1, B). Apparent cases of direct penetration of the outer cork layer of the root were also observed, but they were relatively rare.

The phloem parenchyma, phloem, cambium, xylem, and central portion of the tap root were all readily invaded by *F. avenaceum*. The hyphae proceeded singly through and between the cells, or they aggregated into strand-like masses which ruptured their way through the tissues. Longitudinally, they appeared to progress most rapidly in the vascular tissues

(Fig. 1, C). This probably explains the rapid rotting commonly caused in the central portion of the root (Plate I, D). *F. avenaceum* also appeared to have an advance toxic action, since the cells were often progressively disorganized for some distance ahead of the area where hyphae were visible. Similarly disorganized cells occurred in the dark border formed at the margin of lesions, when active progress of the pathogen ceased (Fig. 1, D).

Physiological Studies

Influence of Temperature

The temperature relations of *Fusarium* spp. were studied in the hope that they might throw some light on the results obtained in the infection experiments. Representative isolates, freshly transferred to plates of potato-dextrose agar, were incubated in quadruplicate at temperatures ranging from −2° to 36° C. Their relative growth was essentially the same in several different series, so the results of only one experiment are presented (Table VIII, Fig. 2).

TABLE VIII

Influence of temperature on growth of *Fusarium* spp. on potato-dextrose agar

Species	Average diameter of colonies in mm. at different temperatures*											
	3°	5°	8.5°	10.5°	14°	17°	20°	24°	27.5°	29.5°	32°	34°
F. avenaceum	0	7	10	15	26	33	37	43	38	21	0	0
F. arthrosporioides	0	6	8	12	19	28	33	38	34	14	0	0
F. culmorum	0	0	0	12	22	36	62	73	73	36	9	0
F. Poae	0	6	8	9	14	21	26	26	18	11	0	0
F. Scirpi var. *acuminatum*	0	0	0	7	12	15	25	30	28	15	0	0

* *Average temperatures in degrees Centigrade for the three days during which the plates were incubated.*

F. avenaceum grew well at a wide range of temperatures, with an optimum at about 24° C., and a maximum at 34° C. It started growth in five days at 1° C., and even grew slowly on frozen agar at −2° C. *F. arthrosporioides* had a similar temperature relation, but it grew more slowly than *F. avenaceum* at all temperatures. *F. Poae* had the lowest optimum and maximum temperatures of any species studied, namely 20° to 24° C., and 32° C., respectively. The minimum for this species was about −2° C.

The other two species developed best at relatively high temperatures. *F. culmorum* had a minimum at 3° C., and grew very slowly at temperatures below 10° C. This species made very rapid growth between 15° and 30° C. It had an optimum at 24° to 27° C., and a maximum at 34° to 36° C. *F. Scirpi* var. *acuminatum* also failed to grow at a freezing temperature, but it did develop slowly at 3° C. Best growth of this variety occurred at 20° to 28° C., with an optimum at 24° C., and a maximum at 34° C.

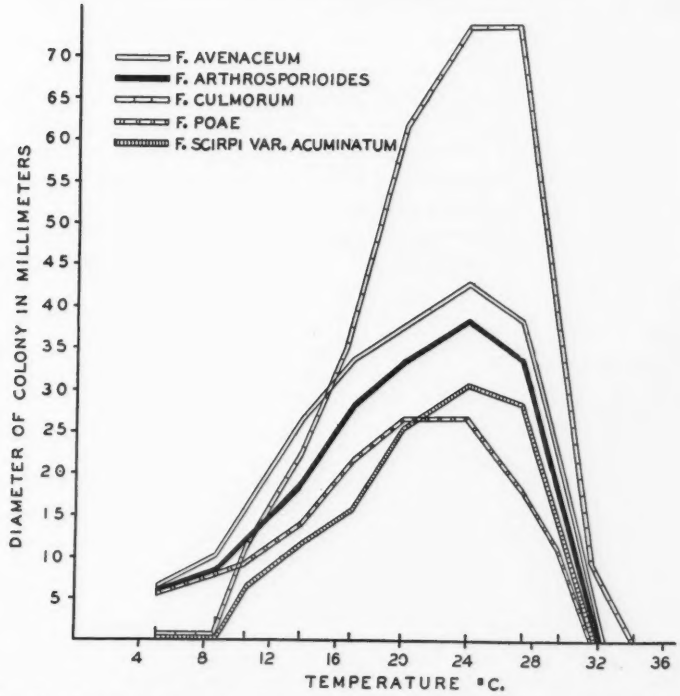


FIG. 2. *Growth of Fusarium spp. on potato-dextrose agar, after incubation for three days at temperatures ranging from 5° to 34° C.*

INFLUENCE OF HYDROGEN ION CONCENTRATION

Fusarium spp. were grown on buffered agar and liquid media adjusted to a wide range of pH values by the methods previously described (5). Their growth on Czapek's synthetic agar is typical of the results obtained (Table IX).

TABLE IX

INFLUENCE OF HYDROGEN ION CONCENTRATION ON GROWTH OF *Fusarium* SPP. ON CZAPEK'S SYNTHETIC AGAR

Species	Average diameter of colonies in mm. at different pH values									
	2.8	4.0	4.6	5.1	5.8	6.2	6.9	7.7	8.4	9.5
*F. avenaceum**	37	70	71	70	72	50	53	60	51	47
*F. arthrosporioides**	39	65	65	64	67	54	58	62	52	49
F. culmorum†	15	48	51	53	58	55	60	55	54	29
*F. Poae**	23	53	55	57	58	56	56	56	56	47
F. Scirpi var. *acuminatum**	12	45	49	50	33	44	45	46	45	26

* *Incubated for six days.*
†*Incubated for three days.*

All species studied grew at pH values ranging from 2.8 to 9.5, but they developed particularly well in the alkaline range. With *F. avenaceum* and *F. arthrosporioides*, the best growth occurred at pH 5.8 and pH 7.7 in potato-dextrose and Czapek's synthetic solutions, and on Czapek's agar (Table IX). There was distinctly less growth at pH 6.2, indicating an isoelectric point in that region. Further evidence of this was obtained in spore germination studies where 90% of the spores germinated at pH values of 5.2 and 6.8, while only 30% germinated at pH 6.0.

More variable results were obtained with the other species studied. *F. culmorum* grew best at pH values of 5.8 and 6.9 on Czapek's agar and liquid media, but had only one optimum, at pH 6.3, on the potato-dextrose media. Best growth of *F. Poae* occurred at pH 6.2 on Czapek's media, and at pH 5.2 on potato-dextrose media. In all media employed, *F. Scirpi* var. *acuminatum* grew best at pH 5.1 and at pH 6.9 to 7.7, with less growth at pH 5.8. Growth of *Fusarium* spp. generally tended to make the liquid media more alkaline.

The ability of these species of *Fusarium* to grow at such a wide range of hydrogen ion concentrations indicates that they should be able to thrive in most cultivated soils. They will probably develop best in soils with the neutral or slightly alkaline reaction suited to growth of alfalfa and sweet clover.

Influence of Carbon Dioxide

There is a possibility that poor soil aeration and consequent accumulation of carbon dioxide around the roots in the early spring may be factors in increasing the susceptibility of alfalfa and sweet clover at that time to attack by *F. avenaceum* and other root-rotting fungi. Lundegårdh (9) found that wheat seedlings were more severely attacked by *Fusarium* spp. when exposed to various concentrations of carbon dioxide than in ordinary air. He suggested that a slowly melting snow covering or a high water content might increase the carbon dioxide content of the soil. During the present investigation an opportunity was not afforded for a study of these soil relations, but experiments were started on the influence of carbon dioxide upon the growth of *Fusarium* spp. in pure culture.

The containers used in these experiments consisted of two coffee tins, of one pound capacity, soldered together and made airtight. Two outlets were provided in the form of short tubes soldered near the top and bottom of each container. A toy balloon of good capacity was sealed on the lower tube to allow for expansion of the gases. Freshly transferred plate cultures, in triplicate, were used. The lid of each plate was lifted slightly by means of an iron wire placed inside. When ready, the plates were stacked in a wire frame and lowered into the container, which was then completely sealed. Carbon dioxide from a commercial tank, measured by water displacement in a burette attached to a levelling bulb, was forced into the container through the upper connection. The previously calculated air content of the container and connections determined the amount of carbon dioxide which it was neces-

sary to add to produce the required concentration. Atmospheric pressure was maintained through expansion of the balloon and, when necessary, a second balloon was sealed on the top connection. Proper mixture of gases was maintained by a gentle pressure applied alternately to the two balloons at regular intervals. Control cultures were placed in similarly sealed cans containing ordinary air. Atmospheres with a carbon dioxide content ranging from normal to 20% were employed. Most of the experiments were conducted at room temperature, but one set of containers was incubated at 8° C. to determine the possible influence of temperature. The data from one experiment (Table X) are typical of those obtained.

TABLE X

INFLUENCE OF CARBON DIOXIDE CONCENTRATION ON GROWTH OF *Fusarium* SPP. ON POTATO-DEXTROSE AGAR

Species	Average diameter of colonies in mm.							
	Room temperature						8° C.	
	Air	CO_2, %					Air	CO_2, %
		2.5	5	10	15	20		15
F. avenaceum†	52	49	49	51	51	49	33	29
F. arthrosporioides†	40	38	36	38	36	39	30	22
*F. culmorum**	90	90	86	85	83	79	46	27
F. Poae†	28	29	27	28	27	23	17	14
F. Scirpi var. *acuminatum*†	42	42	39	40	34	30	22	13

* *Incubated for three days.*
† *Incubated for seven days.*

At room temperature, carbon dioxide concentrations up to 20% had little or no effect on the growth of *F. avenaceum* and *F. arthrosporioides*. These species were, however, slightly retarded by 15% carbon dioxide at 8° C. Growth of *F. Poae* was also very slightly retarded by the higher concentrations of carbon dioxide. On the other hand, *F. culmorum* made consistently less growth as the carbon dioxide concentration of the atmosphere was increased. At 8° C., 15% carbon dioxide markedly retarded the growth of this species. Atmospheres containing 15 and 20% carbon dioxide also had a distinct retarding influence on *F. Scirpi* var. *acuminatum*.

These results are in general agreement with those reported by other workers. Brown (2) noted that carbon dioxide had a greater retarding effect on spore germination and growth at a low temperature than at a high temperature. He suggested that this was partly due to the increased solubility of carbon dioxide in water at low temperatures. Fellows (6) found that growth of *Ophiobolus graminis* was slightly retarded by 18% carbon dioxide, the highest concentration employed. With *Phymatotrichum omnivorum*, Neal and Wester (10) reported that growth was retarded only by concentrations of carbon dioxide greater than 25%. Lundegårdh (9) did not observe any

retarding influence of carbon dioxide on the growth of *F. avenaceum*, *F. culmorum*, and other species of *Fusarium*, but 7% was the highest concentration which he employed. Hence, it appears as if relatively low concentrations of carbon dioxide have little, if any, effect on the growth of *Fusarium* spp. It seems doubtful whether concentrations as high as those which retarded growth in the present study will occur under natural conditions in the soil.

Discussion

Although the species of *Fusarium* studied herein are apparently not as prevalent as certain other pathogens, they do contribute to the root-rot damage suffered by alfalfa and sweet clover in Alberta. The closely related species *F. avenaceum* and *F. arthrosporioides* appear most important, since they occur commonly, and can cause severe damage both in the early spring and during the summer. *F. culmorum* is very virulent in the summer, but it is apparently incapable of attacking the roots in the early spring. The relatively light infection caused at all times by *F. Poae* and *F. Scirpi* var. *acuminatum* indicates that they are weak parasites or saprophytes on roots of alfalfa and sweet clover. This is further suggested by the frequent association of these species with a more virulent pathogen. A final evaluation of the relative importance of *Fusarium* spp. as root parasites of alfalfa and sweet clover in Alberta must await further study.

The relative virulence of *F. avenaceum*, *F. arthrosporioides*, and *F. culmorum* under different conditions appears to be closely correlated with the temperature relations of these species. Since *F. culmorum* was unable to grow at temperatures near freezing, it did not attack roots of alfalfa and sweet clover in the early spring. On the other hand, *F. avenaceum* and *F. arthrosporioides* grew well at a wide range of temperatures, and caused heavy infection in the early spring, as well as during the growing season. These species developed best at about 24° C., while *F. culmorum* had an optimum at 24° to 27° C. This probably explains why *F. culmorum* usually caused slightly higher infection than the other species during the summer, but became less pathogenic with lowering of the soil temperature towards fall. Further evidence of this relation of temperature to infection was obtained in the greenhouse studies, where *F. avenaceum* caused more infection at 24° C. and lower temperatures than at 27° C., while *F. culmorum* did not attack the plants at low temperatures, but was highly virulent at 18° to 27° C. These results are in general agreement with those reported by other workers. Simmonds (15) found that *F. culmorum* from oats had an optimum of 24° to 28° C. for growth in pure culture, and produced a greater degree of infection at 18° to 30° C. than at 8° to 15° C. Bennett (1) concluded that *F. culmorum* was better suited to high temperatures than *F. avenaceum*.

A singular lack of specificity was shown by the isolates of *F. avenaceum*, *F. arthrosporioides*, and *F. culmorum* obtained from alfalfa and sweet clover roots, since they also attacked roots of *Trifolium* spp. and seedlings of wheat, oats, and barley. Furthermore, certain isolates from the cereal crops were

pathogenic on roots of alfalfa and sweet clover. Further study of these and other isolates is necessary, since Tu (16) found two physiologic races of *F. avenaceum*, and three of *F. culmorum* among the forms causing head blight of cereals. However, the present results indicate that the use of legumes or cereals in crop rotation may have certain limitations in reducing the root-rot damage of either crop caused by *Fusarium* spp. Moreover, the fact that isolates of *F. avenaceum* from *Vicia americana* were pathogenic on roots of alfalfa and sweet clover suggests that wild hosts in virgin soil may harbor and increase pathogenic species of *Fusarium*, which may subsequently menace legumes or other cultivated crops.

Acknowledgments

The writer is especially indebted to Dr. G. B. Sanford, Pathologist-in-Charge, Dominion Laboratory of Plant Pathology, Edmonton, Alberta; and to Dr. E. C. Stakman, Chief, Department of Plant Pathology, University of Minnesota, for helpful advice and criticism throughout this investigation. He also wishes to thank Dr. H. W. Wollenweber, Biologische Reichsanstalt, Berlin-Dahlem, Germany, Dr. Louise Dosdall, University of Minnesota, and Dr. W. L. Gordon, Dominion Rust Research Laboratory, Winnipeg, for assistance in the mycological studies; Mr. G. M. Tosh of this laboratory for help with the photographs; and the Dominion Department of Agriculture, Ottawa, for leave of absence which enabled him to carry out a portion of these studies at the University of Minnesota.

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HYBRIDIZATION OF *TRITICUM* AND *AGROPYRON*

III. CROSSING TECHNIQUE[1]

By L. P. V. Johnson[2] and A. McLennan[3]

Abstract

Crossing technique is discussed with respect to coincidental flowering of parental plants, emasculation, bagging, tagging, marking, collection of pollen and pollination.

Data are presented which demonstrate that stigmas of emasculated wheat florets retain their receptivity, and may be successfully pollinated, a week or more after the normal time of flowering. It is also shown that wheat and *Agropyron* pollen may be stored under room conditions for a day or two without appreciable reduction in viability. These points are discussed in relation to crossing technique.

Introduction

During the past three years the authors have been engaged in research that involves the crossing of *Triticum* and *Agropyron*. Since the first year's results were reported (1) the work has been extended to include nine *Triticum* species and sixteen *Agropyron* species, and has progressed to a point where about seventy thousand florets have been emasculated and pollinated. In nearly all crosses *Triticum* has been the maternal and *Agropyron* the paternal parent. Crossing technique, based on this experience, is discussed here with two ends in view: first, to give workers directly interested in the hybridization of *Triticum* with *Agropyron* a more detailed account of the technique than was previously reported; and, second, to give general information, suggestions, and hints that may be generally applicable to hybridization in the *Gramineae* and perhaps in other families. It is assumed that the reader is an experienced hybridizer. Readers who desire more complete accounts of crossing technique than that given here are referred to the work of Hayes and Garber (3, pp. 120-129) for crops in general, to that of Florell (2) for cereals, and to that of Jenkin (4) for herbage grasses.

Obtaining Coincidental Flowering of Parental Material

It is sometimes difficult to make the flowering periods of parental species coincide. In crosses involving two annual forms a number of successive sowings of one of the parents is all that is necessary, and when the hybridizer is familiar with both parents the necessary adjustment may be made by single sowings. Where winter annuals, biennials or perennials are involved, however, more difficulty may be experienced.

[1] *Manuscript received September 7, 1937.*
Contribution from the Division of Forage Plants, Dominion Experimental Farm, Ottawa, Canada. This contribution forms a part of a co-operative investigation on the hybridization of Triticum and Agropyron, undertaken by the Dominion Experimental Farm and the National Research Council of Canada.

[2] *Research Assistant.*

[3] *Graduate Assistant.*

The following conclusions and recommendations are based on experiences in the manipulation of the time of flowering of winter wheats and perennial *Agropyrons:*

(*1*) Practices and treatments, such as different times of sowing, cutting-back, etc., carried out during the previous year do not appreciably change the time of flowering.

(*2*) Cutting-back of wheats and grasses in the spring has practically no effect on the time of flowering, but may be very detrimental to subsequent growth.

(*3*) Midwinter sowing of winter wheat in the greenhouse, followed by exposure of the resulting plants to moderate cold in the late winter or early spring, and transplanting to the field as early as possible, has been reasonably successful. This practice, however, has the disadvantage of being laborious, requiring greenhouse space, and of producing, in our experience, a somewhat poor stand in the field, due probably to the wet, unfavorable, soil conditions generally existing at the time of early spring transplanting.

(*4*) The use of vernalized seed is believed to afford the most satisfactory as well as the simplest means of controlling the time of flowering in winter-annual, biennial and perennial plants. For early plants (of winter wheat in our case) the seed should be vernalized in late winter and started in the greenhouse. For later plants the seed should be vernalized just prior to sowing, which should be done at about the time of the earliest spring wheat sowings. It is often characteristic that a stand from vernalized seed will head over a considerable period of time, an advantageous condition for crossing work. In our experience winter wheats grown from vernalized seed sown in the first week of May have provided a continuous supply of heads for emasculation throughout July.

(*5*) It should be borne in mind that any of these practices may be more or less detrimental and that, since the paternal parent should be favored culturally in order that there may be an abundance of pollen, it is preferable that flowering-time manipulations should be practised upon the maternal parent.

Emasculation

Efficient emasculation involves the complete removal of unbroken anthers from the floret in such a way as to minimize injury, direct or indirect, to the female reproductive organs, and at a time known to be approximately a given number of days in advance of normal flowering. The accomplishment of these requirements is almost entirely a matter of developing skill in the actual operation and in the observation of the proper stages in the plant. There are, however, a number of details about which something useful may be said.

It is usually necessary to adapt standard forceps to emasculation work by grinding or filing the points and by altering the tension. We prefer a straight type with corrugated handles and a point approximately 2 mm. wide. The points are ground down in long, tapering fashion until they are 1·0 to 1·5 mm.

in width and, when examined in sideview, show a nearly straight taper to a fairly sharp point. The tension is usually too strong in standard forceps. This may be weakened by inserting a tapering penholder near the fusion of the handles, forcing them apart, and then bending the handles slightly by pressing the points toward each other with the fingers. A very weak tension is perhaps even more tiring than one too strong. It may be corrected by spreading the handles. The correct tension is one that permits a firm, steady grip without closing the points, yet permits closing the points with a slight pressure.

The standard, straight-bladed, sharp-pointed, dissecting scissors are perfectly adapted to the removal of spikelets, clipping of awns, etc.

Each operator should have a comfortable stool about a foot high, containing a drawer or compartment for instruments, supplies, etc. A folding camp stool to which a suitable pocket had been attached has proved to be very comfortable and convenient.

We usually pollinate wheat florets three days after emasculation, and for this interval select heads the most mature florets of which appear to be two days removed from flowering. The extra day permits most of the less mature florets to become receptive before pollination. The time to emasculate is determined by two factors: the anther must not have matured to the point where there is danger of dehiscence in removal, and the time of flowering must not be too far away to permit a reasonably accurate estimation of the date.

The number of spikelets and florets to be removed from the spike will depend upon circumstances. Ordinarily maximum efficiency is attained if the extreme tip spikelets and a somewhat larger number of extreme basal spikelets are removed, together with the central florets of the remaining spikelets. The range of flowering time in the florets retained should cover only a day or two; ordinarily the optimum practical condition is to have the more mature florets become receptive a day prior to pollination, and the remainder of the florets becoming receptive at the time of or just prior to pollination. If, because of a dearth of material, it is important to cross a maximum number of florets from each head, practically every spikelet (comprising the lateral and probably the larger central florets in most of the spikelets) may be used. Since the range of flowering time in such spikes may cover several days it is advisable to pollinate two or three times, or at least to delay pollination until most of the later florets have become receptive. (While the preceding discussion is based upon the spike-type inflorescence, the points brought out are also more or less applicable to the panicle-type inflorescence.)

Bagging, Tagging, Marking

The glassine envelope is probably the best form of bagging. Glassine paper, while pollen-tight, permits sufficient passage of air and moisture to provide a favorable environment for the enclosed head. Cellophane envelopes, on the other hand, are highly unsatisfactory as they do not permit sufficient

aeration and evaporation. A common mistake in the use of the glassine envelope is to have it far too large. We have been using an envelope size $6 \times 1\frac{1}{2}$ in. on wheat and have found it perfectly satisfactory. (A common size for wheat seems to be $7 \times 3\frac{1}{2}$ in.) The use of large envelopes causes the loss of a great many heads through breakage from wind and rain.

String tags are used to label the heads and are placed well down on the stem to prevent beating about in the wind. It is important that writing should be done with a sharp, medium hard (H or 2H) pencil, using sufficient pressure to make very distinct marks.

Where heads are emasculated as they reach the right stage on successive days and where this is continued over a period of several days, a great deal of time may be saved during pollination by a mark that will indicate at a glance the heads emasculated on a given day. For this purpose we use colored strings, 6 to 8 in. long, for fastening the glassine envelopes to the emasculated heads, a different color being used each day. Thus, when pollinating, we can find immediately the heads emasculated three days previously, even though they are distributed among several hundred other bagged heads. When the envelopes are replaced after pollination the colored strings are replaced by white ones. Since we usually pollinate three or four days after emasculation, only four colors are necessary (though other colors are held in reserve in case pollination is delayed without a corresponding delay in emasculation). Various biological stains are used to dye the strings, among the more successful being: orange G (1% aqueous solution), methyl blue (1% aqueous solution), and acid fuchsin (1% dissolved in 70% ethyl alcohol).

Duration of Stigmatic Receptivity

It is apparently a common belief among hybridizers that the stigma retains its receptivity for only a very short period, perhaps for a few hours or a day or two, depending on conditions. As far as is known no data have previously been published on the period of time over which stigmas retain receptivity, although Florell (2) mentions obtaining good seed sets six to eight days after emasculation in wheat crosses.

The data presented in Table I serve to indicate the duration of stigmatic receptivity in emasculated winter and spring wheats as determined by the relative seed sets upon pollination with *Agropyron glaucum*. Emasculation was performed two or three days before normal flowering time. All spikes were cut down to 20 florets (the two lateral florets of five central spikelets on either side of the rachis), which were in all probability within one day of being at the same stage with respect to flowering time. Hence, it is believed that all stigmas should have been receptive within three or four days after emasculation. Therefore, the data in Table I demonstrate that, under the conditions of the experiment, unpollinated stigmas of emasculated wheat florets remained highly receptive and functional for at least six or seven days after the time of normal flowering.

TABLE I

COMPARISON OF NUMBERS OF HYBRID SEEDS SET IN A SERIES OF WHEAT VARIETIES POLLINATED WITH *Agropyron glaucum* AFTER ELAPSED PERIODS OF FROM TWO TO ELEVEN DAYS AFTER EMASCULATION

Maternal parent (wheat)	2-3 days			4-5 days			6-7 days			8-9 days			10-11 days		
	Florets	Seeds	%	Florets	Seeds	%	Florets	Seeds	%	Florets	Seeds	%	Florets	Seeds	%
Kharkov	—	—	—	60	4	**6·66**	460	163	**35·43**	40	28	**70·00**	40	24	**60·00**
White Odessa	140	43	**30·71**	220	10	**4·55**	660	120	**18·18**	140	0	**0·00**	—	—	—
Dawson's G.C.	—	—	—	140	52	**37·14**	460	88	**19·13**	60	0	**0·00**	40	14	**35·00**
C.D. 1435	—	—	—	300	33	**11·00**	400	55	**13·75**	80	3	**3·75**	—	—	—
Minhardi	—	—	—	100	8	**8·00**	500	91	**18·20**	—	—	—	—	—	—
Secalotricum	—	—	—	140	6	**4·29**	460	86	**18·70**	—	—	—	—	—	—
Minturki	—	—	—	100	5	**5·00**	640	8	**1·25**	60	3	**5·00**	—	—	—
Lutescens 0·329	100	2	**2·00**	140	2	**1·43**	480	3	**0·63**	—	—	—	—	—	—
Lutescens 0·62	180	2	**1·11**	560	6	**1·07**	840	44	**5·24**	—	—	—	—	—	—
Mindum	120	20	**16·67**	460	23	**5·00**	660	30	**4·55**	—	—	—	—	—	—
C.A.N. 1835	200	2	**1·00**	460	5	**1·09**	660	4	**0·61**	—	—	—	40	0	**0·00**
Vernal emmer	80	0	**0·00**	80	14	**17·50**	60	0	**0·00**	—	—	—	—	—	—
Combined data	820	69	**8·41**	2,760	168	**6·09**	6,280	692	**11·02**	380	34	**8·95**	120	38	**31·67**

During the time of the experiment the temperature was warm to moderately hot, while the humidity was at all times moderately high. It is believed probable that the period of stigmatic receptivity would be considerably shorter under extremely hot, dry conditions such as are experienced on the prairies.

With one exception, no pollinations were made later than 11 days after emasculation. The exception was one 20-floret spike of Minturki pollinated 15 days after emasculation. No seeds were set. Florell (2) states that in rare cases hybrid seed was obtained from pollinations made 23 to 25 days after emasculation.

The fact that, under favorable conditions, stigmas may remain highly receptive for a week or more has direct bearing on crossing technique. It is the common practice to pollinate when the first stigmas in the head become receptive; but, since the florets of an emasculated head practically always have a range of a day or two in the time at which they become receptive, and since it is doubtful that dehisced pollen may remain viable for more than a few hours in the floret, it would appear advisable to delay pollination until all florets have become receptive. This is in accord with our practice of emasculating two days (estimated) before flowering, but pollinating three days after emasculation.

Another instance when delayed pollination would be advisable is in crosses involving similar varieties in which selfed seed cannot be distinguished from crossed seed and, more particularly, where the respective characters are not sufficiently differential to distinguish selfed from hybrid plants in the F_1. In such crosses one could eliminate selfed seeds arising from contamination at the time of emasculation by delaying pollination until the seeds had begun to form, indicating that self fertilization had occurred.

Collecting Pollen, Pollination

In our hybridization work with perennial grasses and cereals the pollen is commonly collected in a more or less dehisced condition using 600 or 800 cc. beakers. The use of beakers is to be recommended for a number of reasons; they are handy for collecting, pollinating and storing; they permit adequate aeration of collected pollen, thus preventing "clumping" of pollen grains; and they enable one to pollinate a maximum number of heads with a given amount of pollen. Aeration of pollen, particularly when many anthers are present, is very important under conditions at Ottawa. Pollen grains and anthers transpire sufficiently to increase the humidity to the point where, unless there is adequate aeration, the grains will stick together in clumps of perhaps several hundred. Clumped pollen gives very poor results. Even in open beakers we try to keep the pollen well dispersed by letting it adhere as much as possible to the sides of the beaker when collecting, for, except on days of particularly low humidity, the concentration of pollen in the bottom to any depth will cause clumping. Glumes and anthers falling into the beaker are removed at once, since they give off moisture which may cause clumping. Rainy or excessively humid days are very unfavorable for pollination work.

When collecting pollen from *Agropyron* species artificial stimulation of flowering by "stroking" was commonly practised. (The stroking method consists in stimulating heads which are nearly ready to flower by a few upward strokes with the fingers. Within a few minutes after such treatment the glumes will spread apart and the anthers extrude.) These species characteristically flower very abruptly and over a very short period each day. Since one or two workers were obliged to collect from several species, often in different fields, it was necessary to stimulate flowering artificially some time before flowering would have naturally occurred in order that the rounds could be made successfully. This practice was also useful when collecting from a single species as it made waiting for pollen unnecessary and prevented the possibility of not being on hand when flowering occurred. Pollen is collected by inserting the heads with extruded anthers into the mouth of the beaker where they are rolled or shaken by the fingers to burst or dislodge the anthers.

It is not advisable to induce flowering artificially at a time or under conditions too far removed from the normal. It is often possible to cause anther extrusion several hours before normal flowering time, or on wet, cool days; but pollen thus obtained is sticky, has a tendency to clump, and is of doubtful value in pollination.

Pollination is a more critical operation than emasculation, in that the pollen itself is subject to many conditions which may be, and often are, adverse. The success attending pollination on different days may vary from a negative result to a nearly perfect seed set, even though the same methods and plant materials are used. It is known that such factors as very high humidity, premature stroking, insufficient aeration of pollen, etc., contribute greatly to decreases in crossing success; but it is far from possible fully to account for the differences observed. In short, one cannot be to any degree certain that a given pollination represents a maximum opportunity for fertilization. Perhaps a pollination made on another day, under apparently identical conditions, with a different collection of pollen from the same species, would provide a much better opportunity. Since pollination is ordinarily much easier than emasculation, it may be better, therefore, for an operator to emasculate fewer heads and take time to pollinate all heads two or three times, on successive days or at two-day intervals. We have practised repeated pollinations to a considerable extent and have observed improved seed sets as a result.

Repeated pollinations are also useful in providing fresh pollen for late maturing stigmas which may not have been receptive at the time of the first pollination. Jenkin (4) practised repeated pollinations on certain grasses, especially for this reason. In our own material, however, we feel that the value of repeated pollinations is related in the main to pollen rather than to stigmatic factors.

For applying pollen to the stigmas, sable hair brushes, such as are used for water color painting, have been found to be perfectly satisfactory. We use Winsor and Newton's (England) Series 16, Numbers 1 or 2, which have pointed brushes about 11 mm. long and 1·5 mm. in diameter at the base.

The pollen dust is merely brushed on to the stigmas in sufficient amounts to be noticeable on the brush, one or two florets being pollinated with each brushful. Where there is an abundance of pollen it is probably advisable to imitate nature in being very lavish; but where pollen is scarce it should be used sparingly in order that a maximum number of heads may be pollinated. If the stigmas are not exposed, the glumes may be spread apart by the tip of the brush handle (which should be cut wedge-shaped for this purpose) and held apart with the fingers while pollen is applied to the exposed stigmas.

Methyl alcohol is used for sterilizing beakers and brushes. This chemical is completely effective and, since it evaporates very quickly, there is practically no delay as a result of sterilization.

Storage of Pollen

From time to time in our hybridization work there have arisen situations in which a temporary lack of pollen has emphasized the need for information on the possibility of some simple way of storing pollen for a few days. A difficulty of this kind frequently arose because of afternoon showers which more or less prevented pollination while permitting emasculation, done in the forenoon, to proceed unhampered, resulting in a disruption of our schedule and a superabundance of emasculated heads. We were interested in storing surplus pollen collected on bright days to be used for morning pollination when the number of emasculated heads became excessive. This, ordinarily, would entail storage for only one to three days. Since we could find no literature on the point, an experiment was carried out to test the possibility of such storage.

Pollen of *Agropyron elongatum* and of *Triticum turgidum* was collected on three successive days and stored in beakers under room conditions in a ventilated cabinet. On the fourth day all three stored collections of each kind of pollen, together with fresh check collections, were applied to florets which had been emasculated three days previously. The seed-set results of this experiment are summarized in Table II.

From the results obtained it would appear safe to conclude that pollen of wheat and of *Agropyron* may be stored under room conditions for a day or two without undergoing any great reduction in viability. There is, however, a marked decrease in the number of seeds set when pollen stored three days was used, which indicates that a decrease in viability may begin at about the third day of storage.

In discussing the viability of stored pollen there arises the question of how long dehisced pollen may be expected to remain viable on immature stigmas in the floret. It seems doubtful that pollen would remain viable in an ungerminated condition for any appreciable length of time on the stigma owing to moisture and other germinative factors existing in the floret. Our own observations and those of Florell (2) demonstrate the fact that in certain crosses seed may be produced by pollinating immature stigmas; but it is believed that this is due to the more or less immediate germination of pollen

TALE II

RELATIVE VIABILITY OF FRESH POLLEN AND OF POLLEN STORED FROM ONE TO THREE DAYS AS INDICATED BY SEEDS OBTAINED WHEN USED IN CROSS POLLINATION

Cross	Pollen	Fresh			Stored 1 day		
		Florets	Seeds	%	Florets	Seeds	%
Kharkov × A. elongatum	A. elongatum	142	2	1·41	170	3	1·76
Yaroslav × A. elongatum	A. elongatum	142	3	2·11	168	1	0·60
T. turgidum × T. vulgare	T. turgidum	48	6	12·50	86	4	4·65
Combined data		332	11	3·31	424	8	1·89
Cross	Pollen	Stored 2 days			Stored 3 days		
		Florets	Seeds	%	Florets	Seeds	%
Kharkov × A. elongatum	A. elongatum	146	7	4·79	154	1	0·65
Yaroslav × A. elongatum	A. elongatum	146	3	2·05	156	2	1·28
T. turgidum × T. vulgare	T. turgidum	88	4	4·55	88	0	0·00
Combined data		380	14	3·68	398	3	0·75

and penetration of the pollen tube, rather than to a delay in germination until the stigmas mature. The pollination of immature stigmas however has not, in our experience, been sufficiently successful to be recommended, particularly in wide crosses.

Acknowledgments

The authors are greatly indebted to Dean L. E. Kirk, University of Saskatchewan, Saskatoon, to Dr. F. H. Peto, National Research Council, Ottawa, and to Dr. J. M. Armstrong, Division of Forage Plants, Central Experimental Farm, Ottawa, for valuable suggestions in connection with the preparation of the manuscript.

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CHEMICAL WEED KILLERS

V. RELATIVE TOXICITY OF SELECTED CHEMICALS TO PLANTS GROWN IN CULTURE SOLUTION, AND THE USE OF RELATIVE GROWTH RATE AS A CRITERION OF TOXICITY[1]

By W. H. Cook[2]

Abstract

Substances previously found to be highly toxic when applied to annuals as a spray were also found to be most toxic when added to culture solution. The results by the two methods, however, do not agree as far as the less poisonous chemicals are concerned, certain substances being comparatively more toxic in culture solution than as a spray, and *vice versa*. These discrepancies can be explained by the fact that the dosage in culture solution was varied by adjusting the concentration, whereas in the spraying test it was varied by altering the volume of spray.

The time between treatment and death of the plant generally decreases as the dosage is increased over a limited dosage range, but varies with different chemicals, and appears to be independent of their inherent toxicity.

The size of the plant is seriously reduced at dosages that produce no mortality. The final weight, however, was unsatisfactory as a criterion of toxicity since it was extremely variable. The interfering factors affecting the final weight were taken into account by computing the relative growth rate. The curve relating growth rate and dosage is slightly concave upwards when the dosage is plotted on an arithmetical scale and linear when plotted on a logarithmic scale. The position and slope of the line depends on the chemical. The standard error of duplicate tests increases as the growth rate decreases. On the average, complete mortality occurred at a growth rate of $-2{\cdot}44\%$ per day under the conditions of these experiments, but this is subject to variation due to differences between duplicates, chemicals, and series (plants grown at different times).

Analyses of the culture solutions containing chlorates showed that the amount of chlorate taken up by the plant increased with the concentration present in the culture solution. Nevertheless, only a small, relatively constant proportion of the chlorate present was taken up by the plant at all concentrations.

Introduction

A knowledge of the true toxicity of substances used as weed killers should lead to a better understanding of the factors limiting their efficacy. When a toxic solution is applied as a spray to plants grown on soil, the efficacy of the treatment depends, not only on the inherent toxicity of the chemical, but also on the quantity retained by the leaves, and the detoxicating effect of the soil. It has been shown in previous publications (5, 6, 7) that the quantity of chemical retained by the leaves is dependent on the volume and concentration of the spray solution used, and affects the estimation of the toxicity of leaf sprays to annuals, while the detoxicating effect of the soil is of primary importance in determining the efficacy of herbicides for perennials.

This investigation had as its object the development of a suitable method for estimating the inherent toxicity of chemicals to plants, and its application to certain substances already studied by other methods. Certain results obtained in the early stages of this study indicated that the relative growth rate of the plants might be a better criterion of toxicity than mortality. The relation between growth rate and dosage was therefore investigated.

[1] *Manuscript received September 1, 1937.*
Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as paper No. 18 of the Associate Committee on Weed Control.

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The observed effect of the chemical on the plant is doubtless determined by the concentration of the substance within the plant, although the dosage is generally expressed in terms of the quantity and concentration of the toxic solution applied as a spray, or the concentration of the toxic chemical present in the culture solution. The rate and extent to which different chemicals, or different concentrations of the same chemical, are absorbed, will therefore be a factor influencing the efficacy of a given treatment. An extensive study of this subject was not contemplated, but a number of analyses of the culture solutions to which various chlorates were added permitted an estimate to be made of the extent to which these substances were absorbed.

Materials and Methods

In order to avoid the complications introduced when the toxic solution is applied as a spray, the test plants were grown in culture solution to which different quantities of the chemicals to be tested were added after the plants had developed. This enabled the dosage to be expressed in terms of the concentration of toxic material in the growth medium. It is well known (2, 3, 9) that apparently identical individuals vary considerably in their susceptibility to toxic substances and it seems reasonable to believe that the susceptibility of unselected wild plants such as weeds will be subject to even greater variability. The variability from this source was reduced as far as possible, by using Marquis wheat, grown from carefully selected seed, as the test plant.

The culture solution used consisted of 5 ml. molar calcium nitrate, 5 ml. molar potassium nitrate, 2 ml. molar magnesium sulphate, and 1 ml. molar potassium dihydrogen phosphate, per litre of distilled water. To this solution 1 ml. of 0·5% ferric tartrate was added initially, and every third day thereafter during the initial growth period, and every second day during the final observational period.

The six-week period from germination until the final observation was made may be divided into three phases: germination, initial growth period, and the observational period following treatment. The germination phase consisted of soaking the seeds for 16 hours in culture solution and then placing them on blotters or sawdust soaked with culture solution. These proved to be the best of the several sub-strata for germination tested at the time. The trays containing the seeds and the substratum were then placed in a chamber, covered with a glass plate to provide a high relative humidity, in the greenhouse in which the subsequent tests were made. The temperature was not controlled. Light was excluded for the first few days, and the seedlings were protected from direct sunlight for the remainder of the germination period, which was six days.

At the end of this period the seedlings were selected for size, and transplanted into the experimental jars. These consisted of two-quart sealers previously painted with one coat of black enamel to exclude light, and one coat of white paint to reduce heat absorption. These jars were fitted with

flat corks provided with five holes into which the seeds were secured with cotton batting. The tare weights of the jar and the cork and cotton batting were determined previously so that the weight of the plants and culture solution could be computed at any time from subsequent weighings. In selecting the seedlings, the length of the sprout was the only index of size that could be used without risk of injuring the seedling. All plants within $\pm 5\%$ of the mean height had to be used, as only about 20% of the seedlings fell within these limits.

During the initial growth period of 14 days the weight of the jar and culture solution and the weight of the cork and the plants were determined every three days. The water lost by evaporation and transpiration was made up with distilled water after each weighing. For weighing the plants and corks, a sheet metal guard, having an inverted-funnel shape, was placed on a torsion balance and the cork inserted in the small end. This guard provided a space, at a high relative humidity, for the hanging roots. A drain pan, which did not rest on the balance, was arranged to catch the drip from the roots. Drainage was complete, within the sensitivity limits of the balance, in less than two minutes and the roots did not appear to suffer from exposure to the atmosphere for these short periods.

The observational period extended from the 14th to the 35th day after planting in the jars. On the 14th day, freshly prepared nutrient solution was placed in all the jars to ensure against any deficit in the elements necessary for growth, and the required dosage of the toxic chemical under test was added to the treated jars at the same time. The jars and plants were weighed, as previously described, every two days, and the water loss made up to keep the concentration of the toxic chemical reasonably constant. The final observations on the living plants were made on the 35th day, or 21 days after the addition of the toxic solution. Treatments that resulted in complete mortality within this period were terminated when the plants died. The final observations included separate determinations of the wet weights of the entire plant, all leaf tissue, living leaves, dead leaves, and all roots from each jar. As the wet weight is subject to considerable variation due to variable temperature and humidity conditions, the oven-dry weights of these fractions were also determined for the first few series of treatments. It was then found that the final weight of the plant was a much more variable quantity than the growth rate and, since this quantity had to be computed from the wet weight, further determinations of the oven-dry weights were abandoned.

All the plants were grown in an ordinary greenhouse, subject to the usual variations in growth conditions, during the summer season, *i.e.*, May to September, inclusive. Eight series of plantings were made at different times and these were consequently subject to slightly different growth conditions, as evidenced by the different growth rates subsequently reported. Unfortunately the second series of plants was not as uniform in size as the others at the time of planting and was subsequently affected by a slight

infestation of aphids in the greenhouse, so that the results of only seven series are reported. The dosages tested included 0·005, 0·010, 0·015, 0·025, 0·050, 0·075, 0·125, 0·175, 0·250, and 0·500% of the culture solution on a weight basis. All of these dosages were not used with all chemicals, and a few intermediate doses were employed in some cases. All treatments were made in duplicate on plants grown at the same time, and in a few instances certain treatments were repeated in subsequent series to obtain an estimate of the variation between series. From 6 to 10 untreated controls were used with each series of plants.

The analyses of the chlorate solutions were made by reducing the chlorate with an excess of tenth normal ferrous ammonium sulphate solution, and titrating the excess with standard potassium dichromate solution, using diphenylamine as an internal indicator The ferrous ammonium sulphate was standardized against the potassium dichromate solution before each lot of analyses. In making the determinations the air was removed from the flasks with carbon dioxide, the ferrous ammonium sulphate, and chlorate, solutions added and boiled for 10 min. After cooling and dilution, 15 ml. of an acid mixture, required to provide the conditions necessary for the reaction and titration, was added. This mixture consisted of 150 ml. of syrupy phosphoric acid and 150 ml. of concentrated sulphuric acid in a litre of solution. Three drops of the indicator were then added and the solution titrated. This method was found to be quick and accurate, and has a sharp end point which is not affected by the presence of small quantities of organic matter, as is the permanganate titration.

Estimates of Relative Toxicity from the Certainly Lethal Dose

It was expected that the dosage-mortality results obtained would be capable of being treated by the methods described by Trevan (9) and Bliss (2, 3) and thus allow the effective dosage, and the extent to which it might be in error, to be evaluated. When the experiments were made, however, it was found that, if a given dosage produced complete mortality, the next lower dosage usually killed none of the plants within the test period, although it reduced the growth considerably Under these conditions the certainly lethal dose (C.L.D.) was the only estimate of the relative toxicity obtained, and precise estimates of the extent to which this dosage might be in error were impossible. Duplicate treatments made on plants grown at the same time usually gave the same mortalities, but some variability was evident between series. Arbitrary estimates of the variability are unsatisfactory, and since the chemicals had a decided effect on the size of the plant at dosages that produced no mortality, it appeared that the weight, rather than the mortality, might give a better estimate of the effect of the chemical, and so no attempt was made to determine the dosage-mortality relation more precisely.

In those cases in which the certainly lethal dose of the chemical fell within the dosage range studied, the value obtained is reported in Table I, the chemicals being arranged in order of decreasing toxicity. The relative toxicity

TABLE I

RELATIVE TOXICITY OF CHEMICALS ADDED TO CULTURE SOLUTION, AND APPLIED AS A SPRAY TO PLANTS GROWN ON SOIL

Chemical	C.L.D. in culture soln., %	Relative toxicity when applied as a spray to plants grown on soil	
		Annuals Conc. of spray soln. = 10%	Perennials Conc. of spray soln. = 25 to 20%
Sodium cyanide	0·005	I	III
Sodium arsenite	0·005	II	II
Sodium dichromate	0·010	I	II
Arsenic pentoxide	0·010	I	III
Ammonium thiocyanate	0·015	I	II
Sodium selenite	0·015	I	II
Calcium hypochlorite	0·025	V	—
Nickel sulphate	0·050	VII	—
Sodium arsenate	0·050	IV	—
Sodium sulphide	0·050	III	IV
Potassium permanganate	0·062	VIII	—
Zinc chloride	0·075	II	—
Sodium ferrocyanide	0·075	IV	—
Ammonium chlorate	0·125	II	—
Copper nitrate	0·125	II	IV
Copper sulphate	0·125	V	—
Calcium chlorate	0·250	IV	—
Sodium chlorate	0·250*	II	I
Sodium perchlorate	0·250	V	—
Lead nitrate	0·250	VIII	—
Aluminium sulphate	>0·250	VIII	
Zinc sulphate	>0·175	V	
Calcium chloride	>0·500	VI	
Potassium chloride	>0·500	VII	

** 0·200% gave complete mortality, but as this dosage was not used with the other chemicals, the higher, comparable, value is reported.*

of some of these chemicals, when applied as a spray to annual and perennial weeds, has already been estimated from the C.L.D. under these conditions and reported in earlier publications (5, 7, 8). The grouping of these substances on the basis of their relative toxicity is also given in Table I for comparative purposes, the groups bearing the lower numbers being the more toxic. For annual weeds, substances falling in Groups VII and VIII, and for perennial weeds, those falling in Groups III and IV, are not sufficiently toxic to be of any value as herbicides.

It is evident from the results that the inherent toxicity of a substance, as judged from the culture solution tests, gives no indication of its value as a herbicide for perennials. This is in agreement with earlier results (7, 8)

which showed the efficacy of a chemical for killing perennials to depend on the interactions that take place between the chemical and the soil, as well as on its inherent toxicity.

There is somewhat better agreement between the results obtained in culture solution and those obtained from the spraying tests on annuals. Of the five chemicals found to be most toxic in culture solution, four were classified in Group I and one in Group II in the spraying tests. The remaining less toxic substances are placed in a different order of relative toxicity by the two methods, certain substances being comparatively more toxic in culture solution than as a spray and *vice versa*. Some of these discrepancies can doubtless be attributed to the experimental errors applicable to the estimated C.L.D. by both methods. On the other hand, the two methods differed in that the dosage in culture solution was varied by adjusting the concentration of the toxic substance, whereas in the spraying tests it was varied by altering the volume of a spray of fixed concentration. Where the spraying test showed a given substance to be relatively less toxic than was indicated by the culture solution test, the results can be explained by the discrepancy between the dosage applied as a spray and that retained by the plant, as the divergence between the applied and retained quantities increases (6) as the volume of spray containing unit quantity of toxic chemical increases. Where the culture solution method indicates a lower relative toxicity than the spraying test, the explanation probably lies in the lower concentration of the chemical in the culture solution than in the spray. The concentration of the solution doubtless determines the amount of poison absorbed by the plant (see p. 536) and may also determine its mode of action, *e.g.*, destruction of individual cells or tissues, or transport through the plant.

Although the results of toxicity tests in culture solution are not highly correlated with those obtained in spraying tests, they might be useful when used in conjunction with field tests. In the first place the culture solution determinations can be made more quickly and easily than field trials, and could be used to eliminate substances of low inherent toxicity. Secondly, if the minimum volume of spray required for coverage under field conditions were known, the relative values of the C.L.D. of different chemicals in culture solution might be useful for estimating the best concentration of each chemical to be applied in field tests.

Time Required for Death of Plant Following Treatment

The time required between treatment and death of the plant is important from both the experimental and practical standpoints. Experimentally, the effect of a chemical is determined after a limited observation period, and slow-acting substances may appear to be less effective than those that act more rapidly. Under field conditions a slow-acting poison may permit considerable growth to occur following its application, and this not only complicates the interpretation of the results, but lessens the value of the treatment, as compared with substances that act quickly.

It was hoped in this investigation to obtain a reasonably reliable estimate of the rate at which the different chemicals acted. When the experiments were made it was found impossible to obtain any precise estimate of the time required, owing to the difficulty of determining precisely when the plant was dead. Some plants that appeared to be dead revived when transferred to culture solution free from toxic substances, while others, treated in other ways and apparently alive, died after such a transfer. Nevertheless some of the general features of the results are of interest.

When the elapsed time between treatment and the estimated death point was plotted against the dosage in terms of concentration, the position of the curves for the various chemicals was determined largely by the C.L.D. of the particular chemical and the time required for death at this dosage. Where sufficient data were available, however, it was evident that the curves for all chemicals were of the same general form. The time required for death at first decreased as the dosage increased, and then flattened off at higher dosages, approaching a constant value apparently independent of the dosage. The dosage range employed made it possible to determine this limiting time for 10 of the chemicals. These were as follows: arsenic pentoxide, 4 days; sodium arsenite and sodium selenite, 5 days; copper nitrate and sulphate, 6 days; sodium dichromate, sodium arsenate, and calcium hypochlorite, 8 days; and ammonium thiocyanate and sodium cyanide, 10 days. These limiting times, being independent of the dosage, must be determined either by the rate of absorption of the chemical, or by the rate of the reaction producing mortality within the plant. Comparison of the times given above with the toxicity of the chemicals as judged from the C.L.D.'s reported in Table I indicates that the time required to cause death is independent of the toxicity of the compound.

The dosage–time relation was then studied after reducing these two quantities to a comparable basis for all chemicals. This was done by converting the dosage, in terms of concentration, to the number of C.L.D.'s it represented, and the time in days to a percentage of the time required at the C.L.D. When these quantities were plotted all the curves fell into two groups, the curves for each of the chemicals in the two groups coinciding over the dosage range up to about 2 C.L.D. Beyond that point the curves flattened out at various levels corresponding to the fixed times already reported. The group having the greatest initial slope, and the greatest final reduction in killing time, included only arsenic pentoxide and sodium selenite. All the other chemicals for which adequate data were available fell into the second group. The difference between these two sets of curves, on a comparable physiological basis, supports the earlier conclusion that the rate at which a chemical acts is independent of its inherent toxicity.

Dosage–Weight Relations

It has already been pointed out that sub-lethal doses seldom produced partial mortalities, although they reduced the weight of the plants considerably. The use of shorter dosage intervals might have produced partial

mortalities over a short range, but estimates of the toxicity based on this criterion would have neglected the toxic effect of the chemical as indicated by the reduction of weight at low dosages. The extent to which the wet weight is reduced by sub-lethal doses is shown by the results given in Table II for three of the chemicals. It appeared from these results that the methods and analysis described by Trevan (9) and Bliss (2, 3) applicable to the dosage–mortality relation might actually be applicable to the dosage–weight relation in plants. The use of the weight, rather than the number, of living plants has already been suggested (7), on the basis of practical considerations, for determining the efficiency of herbicides in the field, and other investigators (4) have used the weight of the plants as a criterion of toxicity.

TABLE II

DOSAGE–WEIGHT RELATION

Dosage conc. in culture soln., %	Wet weight of entire plant as percentage of corresponding controls		
	Sodium chlorate	Zinc sulphate	Aluminium sulphate
0·025	62·8	58·2	50·6
0·050	31·2	42·8	21·8
0·075	25·8	28·5	20·3
0·125	14·7	17·3	21·8
0·150	23·1*	—	—
0·175	20·0*	22·8*	18·8
C.L.D.	0·200%	>0·175%	>0·250%

* *Results from a later series under slightly different growth conditions.*

In view of these facts an attempt was made to determine the relation between the dosage and the final weight of the plants. It was evident that the final weight of the entire plant, on either a wet or dry basis, could not yield a sigmoid curve when plotted against dosage, since the final weight of the plant tissue, whether dead or alive, would always have a finite value, dependent on the size of the plant used. The weight of living tissue at the end of the experiment was therefore used in an attempt to establish the relation, but in spite of the large body of data available, the results were too variable to permit a definite conclusion. Part of the observed variability doubtless arose from the rather arbitrary separation of the living and dead tissue. The difficulty of determining when an entire plant is dead has already been mentioned, and this problem is present in greater degree in tissue separations. However, the greater part of the variability was contributed from other sources; namely, the final wet weight of the entire plant, whether treated or not, and this variability was reflected in the weights of the living and dead tissues on both a wet and dry basis. Since this variability could not be attributed to a variation in the susceptibility of the plants to the chemicals, the untreated controls being subject to fluctuations of comparable magnitude, a further study of the dosage–weight relations was abandoned in favor of a study of the dosage–growth-rate relations.

Relative Growth Rate as a Criterion of Toxicity

The relative growth rate, or the increase in weight per unit of weight already attained, as well as per unit of time, takes into account the variable initial weight of the plant, and the rate at which the chemical acts. Making use of the equation $\frac{1}{w}\frac{dw}{dt} = \frac{d \log w}{dt}$, the relative growth rate was computed from the natural logarithms of successive weights. When this was done, it was found that the coefficient of variability of the growth rate was much smaller than that of the final weights. For instance the means and standard errors for the plants in the 10 untreated control jars in the first series were: final wet weight per jar, $68 \cdot 6 \pm 8 \cdot 3$ gm. and the mean relative growth rate over the entire period, $8 \cdot 90 \pm 0 \cdot 33\%$ per day. These values give coefficients of variability of $12 \cdot 1\%$ and $3 \cdot 7\%$ respectively. The relative growth rates for both treated and untreated jars in all series were similarly less variable than the final weights.

The weights of the plants during the first two-week growth period were so small that the growth rate could not be determined accurately, and the weight on the 14th day, when the poison was added, was taken as the initial value. In consequence it was impossible to determine whether the weight differences then observed were the result of slight differences in size that were not detected when the selections were made, or were caused by slight injuries to the seedlings at the time of planting which affected their growth rate during the initial growth period.

Before considering the relation between relative growth rate and dosage, the factors affecting the comparability and variability of the growth rate over the observational period, *i.e.*, 14 to 35 days, must be examined. During this period the plants were weighed every two or three days, which permitted the relative growth rates to be calculated independently for each of these periods. In Fig. I, A the observed growth rates have been plotted against the midpoint of the period over which they were observed, for the untreated controls in the seven series. It is evident that the growth rate varies between series, and within series it varies from day to day, a result attributable to the different growth conditions prevailing at different times. The curves also give some indication of the magnitude of the reduction in growth rate with age.

The variation in growth rate between series shows that valid comparisons of the effect of different treatments cannot be made on plants grown at different times until the effect of the chemicals on plants having different growth rates has been established. Even within series the variation in the growth rates from day to day may introduce a disturbing influence if the treated plants respond to the variable conditions differently than the untreated controls. This possibility was tested by plotting the growth rates of treated and untreated plants in the same series against time. A typical graph of this sort, for the results obtained with sodium chlorate, is shown in Fig. I, B. It is evident that although the mean relative growth rate decreases as the

dosage of poison increases, the growth rate of the treated plants from day to day varies, in most cases, in accordance with the growth rate of the untreated controls. It is of interest to note that not only was this true for treated plants that showed a positive growth rate, but also for the maximum dosage when

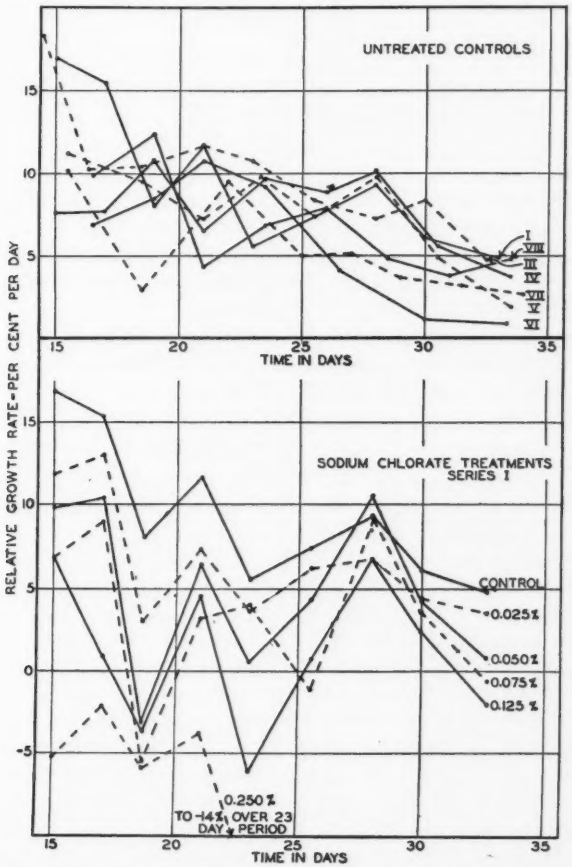

FIG. 1. *Growth rate of untreated and treated plants during observation period.*

the plants had a negative growth rate throughout. The parallel behavior at negative growth rates may be fortuitous, since the small initial weight decreased with time, and the errors in weighing such small quantities of material could easily account for the observed differences.

The steady decrease in growth rate with age of plant did not appear to be a serious complication, If a given treatment did not cause complete mortality at the end of the observation period, the average rates for the treated and

untreated plants would be comparable. Certain treatments, however, killed the plants a few days after the toxic chemical had been added, and in such instances the final weighing was made as soon as the observer was sure that the plants were dead. Here, the comparable value of the growth rate for the control would be that observed over the period between treatment and death of the treated plant, rather than the average over the entire observational period. The difference between the growth rate of the controls over the two periods in question was subsequently found to be small compared with the variability arising from other sources.

The practice of expressing the loss of weight associated with death as a negative relative growth rate may be questioned. The decrease in weight is due largely to the loss of moisture from the dead tissue, and since its magnitude will depend on the weight of the plant as well as on the time, the use of a relative rate appears to be sound. On the other hand it must be recognized that the relative rate of moisture loss from dead tissue may differ considerably from the relative rate of growth of living tissue under similar conditions. Since the plant tissue will dry out continuously, and the death-point of the plant cannot be determined precisely, it is evident that the negative growth rates associated with death will be subject to considerable variability.

Before proceeding to a discussion of the average growth rates over the entire observational period, it is of interest to consider the systematic changes in growth within the observational period for treated plants. In order to do this the growth rates between the initial and succeeding observations were computed and plotted. This procedure "smoothed" the curves and eliminated most of the erratic day-to-day variations. It was then found that treatments with slow-acting poisons, such as the chlorates, behaved somewhat like the untreated controls, in that the growth rate decreased during the first week of the observational period and then remained at a more or less constant value. As the dosage of such chemicals was increased, the curve was changed in position to lower growth rates but retained the same general shape. A dose of 0·2% sodium chlorate caused a continuous loss of weight corresponding to a negative growth rate of about -1% per day throughout the entire period from the second to the seventeenth day after treatment, when the plants were dead. On the other hand plants treated with quick-acting chemicals, such as the arsenicals, suffered weight losses corresponding to continuously decreasing growth rates. Thus 0·01% of arsenic pentoxide decreased the growth rate from 7·0 to 5·0% per day during the first 2-day period while the value over a 12-day period was $-4{\cdot}7\%$ per day. The other chemicals usually showed a behavior between these two extremes, but a few showed evidence of stimulation during the first two or three days that was not evident over longer periods. Some treatments caused a continuously decreasing growth rate, but the plants were not dead at the end of the observational period. Chemicals exhibiting this behavior might have caused complete mortality over a longer period of time, at lower dosages than those reported in Table I.

The average growth rate over the observational period or, when the plants died within this time, over the period between treatment and death of the plants, was used to plot growth-rate–dosage curves. In order that the reported growth rate would not be based entirely on the initial and final weights, the growth rate was computed for both the period between the first and last, and the second and second-last weighings. These two values were then averaged to obtain the growth rate for each jar, and the replications of any one treatment, in any one series, were again averaged to obtain the reported values.

The curves obtained are shown in Figs. 2 and 3, the dosage being expressed in terms of per cent concentration of chemical in the culture solution. Within the dosage range studied, there was no evidence of stimulation over the entire period, all the treatments giving growth rates comparable with, or less than, the untreated controls. The curves are generally quite smooth throughout the range of positive, and for a short range of negative, growth periods. Large negative growth rates frequently showed considerable variability and many of the points obtained at dosages in excess of that required for complete mortality have been omitted from the graphs for the sake of clarity. The curves are usually slightly concave upwards, but sometimes tend to flatten off abruptly at large negative growth rates.

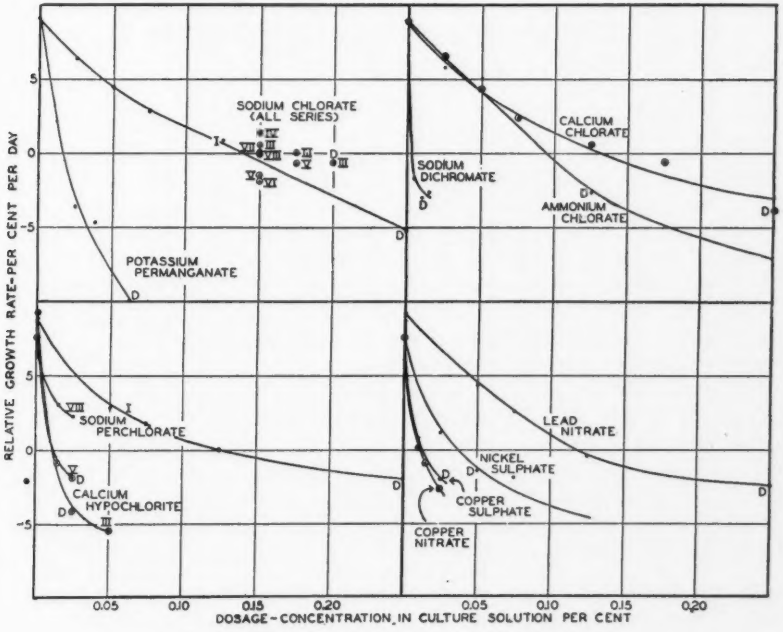

FIG. 2. *Relation between growth rate and dosage.*

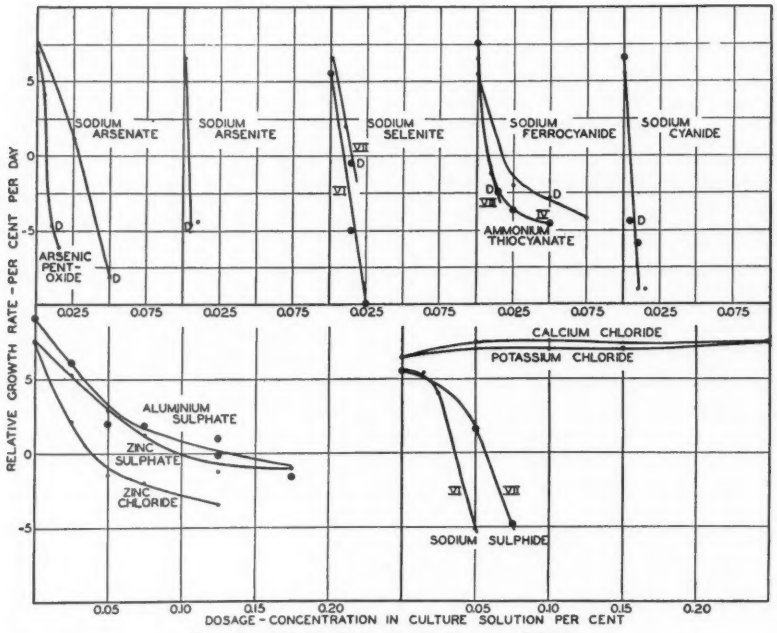

FIG. 3. *Relation between growth rate and dosage.*

Analysis of the dosage–mortality relation (2) indicates that, in many organisms, the effect of the toxic substance increases in proportion to the logarithm of the dosage rather than the dosage itself. It might be expected therefore that a linear relation would exist between the logarithm of the dosage and the relative growth rate. The available data were inadequate to permit this relation to be tested for all the chemicals used, but the results for six substances are plotted on this scale in Fig. 4. It appears from these graphs that the relation is a linear one within experimental error. This result is in agreement with Bateman's findings (1) which show a linear relation between the logarithm of the percentage retardation in growth and the logarithm of the dosage, for a number of organisms. There appears to be no advantage in Bateman's method of expressing the growth obtained with added poison as a percentage of the controls, or avoiding the inverse relation by computing and plotting the retardation instead of the observed growth rate, against the dosage. It appears from Fig. 4 that the position and slope of the lines, for a given species grown under the same conditions, are determined by the properties of the chemical.

It is obvious that treatments that reduce the growth rate compared with that of the controls, will reduce the relative size of the plants, although these growth rates may be positive. Partial mortalities were seldom observed in

these experiments, but the few that did occur fell in the region of positive, but retarded, growth rates. In one instance, a 20% mortality occurred at an average growth rate of 2·5% per day. This was the lowest partial mortality and the largest growth rate at which any plants died in these experiments.

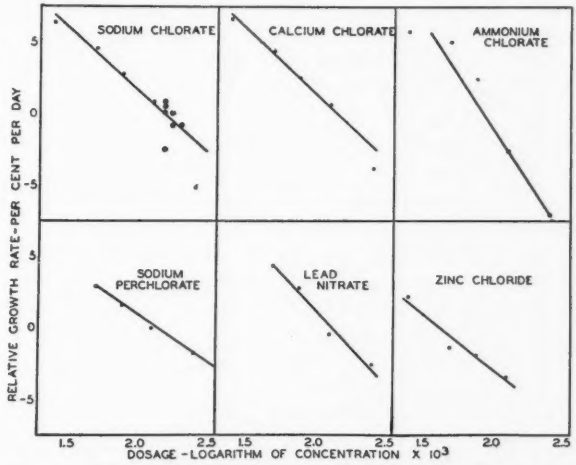

FIG. 4. *Relation between growth rate and the logarithm of the dosage.*

The next point was to determine the growth rate at which complete mortality occurred. On theoretical grounds it would appear that a growth rate of zero should result in complete mortality if the observations were made over a sufficient period of time. In practice, however, the death of the plant cannot be detected until some time after it has occurred. The continuous loss of weight during the interval between the actual and observed death points introduces a bias which brings the observed growth rate into the negative region, and this bias tends to be greater as the time between treatment and death decreases.

In order to determine the growth rate corresponding to complete mortality, the growth rates at the C.L.D. for all chemicals, *i.e.*, the points marked D on the curves in Figs. 2 and 3, were treated statistically. In making these analyses, the results obtained with potassium permanganate and the arsenicals were excluded, since the C.L.D.'s occurred at comparatively high negative growth rates. It was observed that potassium permanganate affected only the roots actually immersed in the solution, so that the plant probably died from mineral starvation rather than from a toxic effect. The high negative growth rates observed at the C.L.D. of the arsenicals are probably the result of inadequate data. Excluding these substances, and those whose C.L.D. did not fall within the dosage range studied, the mean growth rate for all the other chemicals at the C.L.D. was found to be $-2{\cdot}44\%$ per day.

The individual values fluctuate around this mean with a standard deviation of $\pm 1 \cdot 35\%$ per day, but the variance included in this standard deviation is contributed from three distinct sources: the variance between duplicates; the variance between series, since the different chemicals grouped in the above calculation were tested at different times; and the variance between chemicals, if different poisons cause complete mortality at different growth rates. Unfortunately the data were inadequate for a complete analysis of the variance contributed by these three sources, but it was possible to make some estimate of the standard error of duplicates and the variance between series.

In computing the standard error of duplicates the complete results were divided into four groups: (1) controls; (2) treated plants with growth rates above 0% per day but below controls, *i.e.*, in the subnormal growth and partial mortality region; (3) treated plants with growth rates between 0 and -5% per day, *i.e.*, in the range in which complete mortality can be expected; and (4) growth rates less than -5% per day. The values obtained are reported in Table III, from which it is evident that as the growth rate decreases

TABLE III

GROWTH RATE: STANDARD ERROR OF DUPLICATES

Range of growth rates	Degrees of freedom	Standard error, % per day
Untreated controls	43	0·46
Treated plants, growth rates greater than 0% per day, *i.e.*, sub-normal growth and partial mortality range	51	0·60
Treated plants, growth rates 0 to -5% per day, inclusive, *i.e.*, complete mortality range	55	0·86
Treated plants, growth rates less than -5% per day, *i.e.*, complete mortality range but dosage excessive	35	1·16

the standard error of duplicates increases. This result can be partly explained by the fact that the percentage error of weighing increases as the weight decreases, *i.e.*, as the growth rate decreases. It seems likely, however, that the error in the negative growth-rate regions associated with death is also affected by the difficulty of estimating the exact death point of the plants.

The untreated controls differed significantly between series. This, however, is of less interest than the variance between identical treatments in different series. A few tests of this type, corresponding to 19 degrees of freedom, resulted in growth rates between 0 and -5% per day, the range of greatest interest. Analysis of these data showed that the variance between series and chemicals was significantly greater than the variance between the corresponding parallel treatments. Further analysis indicated that different

growth conditions, as indicated by the relative growth rate of the controls, affected certain chemicals in one direction and others in the reverse way, but the data were too few to permit a definite statement. The effect of similar treatments on plants having different growth rates is shown in Figs. 2 and 3, by the points obtained with sodium chlorate in all series, and the curves obtained in different series for some of the chemicals. It is therefore evident that the standard error of duplicates is only a small part of the error involved in estimating the relative toxicity of two chemicals, particularly if these determinations are made at different times.

In conclusion it appears that the relative growth rate is a more useful measure of the effect of chemicals on plants than mortality. Where chemical treatments capable of destroying the weeds entirely are too costly, it may be practicable to apply dosages that will control the weed by reducing the growth rate. Here the relative toxicity of different chemicals should be assessed from the dosage range that causes a reduction in growth rather than from the dosages required to produce complete mortality. Since the relation between relative growth rate and the logarithm of the dosage appears to be linear, over the range of practical interest, the relative toxicity of different chemicals can be readily estimated from the dosages required to produce any given growth rate.

The relative growth rate can also be used for predicting the dosage required to cause complete mortality. It seems likely that this method, when calibrated, would be less time-consuming than determining the mortality directly. When the latter method is used, a considerable period must elapse between treatment and the final observation to ensure that the maximum mortality is obtained, and to ascertain whether or not the affected plants are dead. The error of predicting the mortality from the growth rate appears to arise mainly from the variance between chemicals and between test plants grown at different times, *i.e.*, under different growth conditions, the standard error of duplicates being relatively small. The variable behavior of plants grown at different times is also common to toxicity determinations based on mortality. On the other hand the growth rate method permits a study of the interactions between chemicals and growth conditions, as indicated by the growth rate of the untreated plants, thus providing information as to the possible effect of variable environmental conditions on the efficacy of a chemical.

Absorption of Chlorates from Culture Solutions

Chlorate salts form the basis of many commercial herbicides. Previous investigations (4, 5, 6, 7, 8) have shown that they are quite toxic, and since they are not readily detoxicated in the soil they are effective for the destruction of perennials. It is evident, however, from results presented in this and earlier papers that they act rather slowly as compared with several other chemicals. These considerations led to a few preliminary measurements on the amount of three chlorate salts absorbed from the culture solution at sub-lethal dosages. No analyses are reported on solutions containing lethal

dosages, since the exact time at which the plant died could not be judged accurately, and contact with the dead roots might decompose part of the chlorate salts and yield fictitiously high results for absorption.

The initial concentration of chlorate salts was known from the analysis of the stock solution and the dosage used. No determinations were made during the observational period. Before determining the final chlorate content of the solutions, sufficient distilled water was added to bring the weight up to the original value. Duplicate analyses were made on the solution from each jar, and the results reported in Table IV are based on the

TABLE IV

ABSORPTION OF CHLORATES FROM CULTURE SOLUTION

Chemical	Amount added per jar, gm.	Amount absorbed		Ratio of amount absorbed to final wet weight of plants, %	Ratio of conc. absorbed to conc. in culture soln., %
		gm.	%		
Ammonium chlorate	0·50	0·06	12	0·20	8
Ammonium chlorate	1·00	0·12	12	0·47	9
Ammonium chlorate	1·50	0·06	4	0·46	4
Sodium chlorate	0·50	0·09	18	0·44	10
Sodium chlorate	1·00	0·10	10	0·45	10
Sodium chlorate	1·50	0·16	10	0·90	12
Sodium chlorate	2·50	0·19	8	2·33	20
Calcium chlorate on anhydrous basis	0·43	0·08	18	0·26	16
Calcium chlorate on anhydrous basis	0·87	0·08	9	0·35	10
Calcium chlorate on anhydrous basis	1·30	0·11	8	0·74	11
Calcium chlorate on anhydrous basis	2·17	0·10	5	1·04	10

average of four determinations at each dosage. The duplicate samples taken from the same jar showed close agreement, indicating that the method was satisfactory, but there was frequently considerable variability between the duplicate jars treated in the same way, and this source of variability probably accounts for most of the evident irregularities. In general, the amount of chlorate absorbed increased with the dosage or initial concentration, but on a percentage basis less was absorbed from the more concentrated solutions. Since the weight of the plants decreased as the chlorate concentration increased, the quantity absorbed was expressed as a percentage of the wet weight of the plant. These figures, given in the fifth column of Table IV, are more regular than the others and show the importance of considering the size of the plant in determinations of this sort. In order to obtain an estimate of the effective toxic concentration within the plant, the percentage of chlorate

salt in the plant was plotted against the growth rate, and extrapolated to the growth rate corresponding to 100% mortality. This indicated that a chlorate salt concentration of about 3 to 4% of the wet weight would be required to produce complete mortality. The data were too few to permit a definite conclusion, but the above figure is in agreement with some of the lower values computed from analysis of solutions containing lethal dosages in which the chlorate is subject to decomposition as mentioned previously.

The amount of chlorate salt absorbed and the total amount of water transpired during the observation period was used to calculate the average chlorate salt content of the solution absorbed by the plant. This concentration, expressed as a percentage of the concentration present in the culture solution, appears in the last column of Table IV. These results show that, on the average, the plant absorbed a solution containing only one-tenth as much chlorate as that present in the culture solution. Some of the data obtained suggested that this marked selectivity on the part of the plant may break down as the death point is approached, but this cannot be stated with assurance. This highly selective absorption of chlorates is nevertheless extremely important. In the first place it shows that chlorates must be added considerably in excess of the amount required to kill the plant. Secondly, it shows the effect of dilution on the efficacy of chemicals of this type. It has already been shown (7) that the concentration and quantity of the solutions normally used against perennials in the field act mainly through the soil. Since the free moisture content of soil is subject to great variation, it follows that the efficacy of a given dosage will be subject to large variability from dilution, quite apart from differential losses by detoxication and leaching.

Acknowledgment

The author wishes to thank Mr. A. E. Chadderton and Mr. G. N. Seed, Laboratory Assistants, National Research Laboratories, for technical assistance during the course of this investigation.

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PHYSIOLOGIC CURVE OF RESPONSE TO PHYTOHORMONES BY SEEDS, GROWING PLANTS, CUTTINGS, AND LOWER PLANT FORMS[1]

By N. H. Grace[2]

Abstract

In all plant species tested, increasing concentrations of phytohormones produced responses falling on a physiologic curve from minimum through optimum to maximum which, if exceeded, led to injury and death. Indolylacetic acid, its butyric and propionic homologues, naphthylacetic acid, their salts, and mixtures gave similar results. Treating seeds with hormones incorporated in adsorbent dust stimulated both root and top growth markedly, with less danger of overdosage than in solution treatment. Dosages equivalent to 50 to 250 mg. per acre applied as dilute solutions to soil growing young lettuce and tomato plants covered the optimum range of stimulation to growth. Dust treatment of cuttings proved very convenient and successful in inducing rooting, the plants again showing a wider range of tolerance to dusts than to solutions. Fermentation of sugar by yeast responded to hormone stimulation. Various practical applications are discussed.

Introduction

The physiological activity of the group of chemicals loosely designated by the general term plant growth substances or phytohormones has been established through the work of Kögl, Went, Thimann, Laibach, Zimmerman, Hitchcock and many others. Recent study of the practical utility of these compounds has been related to their ability to cause and hasten root formation by plant cuttings. The results of this work have led to their successful use in plant propagation. While the epinastic or bending response is widely used as a criterion of physiological activity, the magnitude of this response does not give a comparative measure of the root-producing power of the various compounds (7). Among the other responses reported recently are those relating to the effect of heteroauxins on legume nodule formation, and the production of parthenocarpic fruits (3, 5).

It is commonly stated that growth hormones do not increase root development of growing plants; in fact many investigators report an inhibition of root growth. The present writer has found such inhibition to be due usually to overdosage. Plant roots are extremely sensitive even to low concentrations of the active chemical; ordinarily dilute solutions may exert a damaging effect. However, if an exceedingly small amount of phytohormone is applied to the germinating seed or to the plant in a gradual manner, pronounced growth stimulation is usually noted. As hormone dosage increases, growth stimulation also increases until a peak is reached, then falls off to zero and finally below the value for untreated control plants. The results when plotted describe the familiar physiological curve. Such results have been obtained with 3-indolylacetic acid, its homologues, γ-(3-indolyl)-butyric and β-(3-

[1] *Manuscript received October 23, 1937.*
Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa.

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indolyl)-propionic acids, and with 1-naphthylacetic acid, and mixtures and salts of these applied to seeds of many kinds, to growing plants such as wheat, tomatoes and nasturtiums, and to yeast and certain bacteria.

It must not be inferred that all the active chemicals are equally effective. The response varies not only with the hormone, but also with the method of application, and with the species and stage of development of the plant. Some plants are more easily damaged or killed by a particular hormone. Generally speaking, there appears to be least danger of shock when the naturally occurring hormone, indolylacetic acid, is used to root cuttings, though in many cases superior rooting occurs with the synthetic compounds. The rooting of cuttings over a range of hormone concentrations follows the same general type of physiological curve obtained with germinating seeds and growing plants. Another interesting phenomenon frequently observed, when the cut ends of herbaceous plants are placed in suitable dilute solutions of phytohormones, is a marked swelling or increased turgor.

This paper is introductory. Only illustrative results of a wide range of experiments are given in the following sections. Investigations are still actively under way, and more detailed accounts of the various phases, where desirable, will appear in due course.

Hormone Treatment of Seeds

Many workers in this field have reported inhibition of root development when seeds or very young plants are treated with phytohormones. The writer has found that the usual solution methods of applying the hormones, even in dilute concentration, do retard root growth. However, if the seed is treated in such a manner that a small, gradual supply of active material is made available, there is a resulting increase in both root and top growth. In some cases most striking root development results.

Cholodny (2) has reported a substantial increase in yield when oat seed is soaked in hormone solution prior to planting. We have tried this method with oats and have failed to get any increased yield, using the range of concentrations he described. However, our seed was dried after treatment to conform to recognized seed treatment practice.

The salient feature of the method of seed treatment developed in this laboratory is the use of an adsorbent dust carrier. As the treated seed swells and starts to germinate, the adsorbed, adhering hormone is made available gradually. The dust application of hormone chemicals to seeds provides a ready and accurate method of applying definite amounts. The danger of overdosage is substantially reduced. The carrier may be talc, or other suitable dust, or a standard mercurial dust disinfectant. The growth-promoting chemical is intimately mixed with the carrier by a grinding mix or other thorough mixing operation. The amount of chemical added to the carrier will depend on the dosage desired and the rate of application of dust to the seed. In treating cereal crops one half ounce of dust has been applied to a bushel. Treatment of garden and other seeds has necessitated the use of a

very wide range of dust additions. Hormone treatment is expressed in parts, by weight, of the growth-promoting chemical per million parts, by weight, of seed.

Stimulation of Wheat Roots

As illustrative of the root stimulation that may be obtained by seed treatment, a few values follow. Root measurements were carried out on lots of twenty plants from each group of hormone treatments. The plants were grown in sand at 12° C. and were removed for observation 14 days after planting. Using controls treated with the carrier alone, a mercurial disinfectant dust in this case, treatment with 2 p.p.m. indolylacetic acid resulted in a 65% increase in the length of roots. A mixture of 2 p.p.m. of indolylacetic and 2 p.p.m. of naphthylacetic acids effected an increase of 102% in root length. Treatment with 2 p.p.m. of indolylbutyric acid increased root length by 55%. The dry weights of the tops were increased up to 20%.

Stimulation of Barley Roots

Roots of barley seedlings from dust-treated seed, germinated in sand and washed out 14 days after planting, are shown in Plate I, Fig. 1. The four bunches at the left represent successively the control and three increasing doses of naphthylacetic acid. Photographed with the crowns at the same level, the roots indicate the typical physiological curve obtained in all this work. The lowest concentration of hormone (second bunch) represents the optimum in this case, and the treatment accorded the fourth bunch has obviously exceeded the maximum dosage giving positive stimulation. The fifth bunch (last at right) shows the effect of a mixture of naphthyl- and indolyl-acetic acids, totalling 5 p.p.m., to be positive stimulation falling between that resulting from 2.5 and from 12.5 p.p.m. of naphthylacetic acid alone.

Roots of barley seedlings of the same age and from the same planting, but treated with indolylacetic acid, are shown in Plate I, Fig. 2. The results are of the same general nature though not so sharply defined as those in the preceding experiment. Apparently the plants have a wider range of tolerance to indolylacetic acid, though the response to naphthylacetic acid is often more marked.

In both the foregoing experiments, a standard mercurial disinfectant dust was used as the carrier for the hormones.

Soya Bean Roots

Experiments were made with soya beans grown for six weeks in flats of good soil, using both dust and solution methods of seed treatment. In the latter case the seeds were allowed to dry to a condition suitable for planting in the ordinary way, since the experiments had reference to the agricultural applicability of the method. In Plate I, Fig. 3 the first bunch at the left, dust-treated with 10 p.p.m. naphthylacetic acid, shows a more vigorous response than its next two neighbours, dust-treated respectively with 20

and 5 p.p.m. indolylacetic acid. Of the latter two, the higher concentration proved better. The control bunch at the right was treated only with the standard disinfectant dust used as a carrier in the other three cases.

In Plate I, Fig. 4, the left-hand bunch represents the effect of dust treatment with 10 p.p.m. indolylacetic acid (talc being used as the carrier), compared in the next two bunches with solution treatment at 100 and 10 p.p.m. of solution, respectively. The seed was soaked for an hour, then drained and allowed to dry. Comparison of the two solution treatments with the untreated control at the right, shows that damage has occurred in both cases, whereas the lower concentration applied as a dust (first bunch at left) produced increased growth.

Physiological Activity of Salts of Growth Hormone Acids.

In addition to indolylacetic acid and naphthylacetic acid and combinations, a number of other physiologically active substances have been investigated with seeds. Activity is shown by the propionic and butyric acid homologues of indolylacetic acid and by their salts; phenylacetic acid has also been shown to have a measure of activity. There are indications that the salts are particularly effective growth stimulants when applied by this method. An example of this is shown in Plate I, Fig. 5. Both the naphthylacetic acid and its potassium salt (right) caused increased growth of roots and tops, as compared with the control (left) treated with the disinfectant dust carrier only, but the salt was slightly more effective than the acid.

Dust applications of optimum concentrations of phytohormones to seed have increased the dry weight of the tops of month-old wheat plants, grown in soil, as much as 20 and 30%, and of the roots as much as 65%. The rate of emergence of seminal roots is apparently increased by certain concentrations of growth substance. Overdosage represses both root and top growth. While specific reference has been made to field crops in the above, a number of varieties of garden seeds have also been tested and shown to respond in a similar manner.

Hormone Treatment of Growing Plants

The marked response obtained from seeds dusted with hormones suggested the application of small amounts to growing plants. The earlier reports on direct application of phytohormones to plants have failed to give very definite results. Pearse (6) has studied the effect of phenylacetic and indolylbutyric acids, spraying with 0.1% solutions. Recently Greenfield (4) reported fairly marked growth stimulation from the application of solutions of indolylacetic acid. The writer finds that the use of the correct range of dosage gives very definite and clear-cut results, showing good stimulation with optimum additions, and damage with overdosage.

In Plate I, Fig. 6 shows tomato plants which, eight days after seeding in soil, were transplanted to sand moistened with Hoagland's solution (two plants per four-inch pot, in lots of five pots). Thereafter each pot received

50 cc. daily of Hoagland's solution, to which in the lot represented by the centre pot in Fig. 6, 1/100 p.p.m. and, in the right hand pot, 1 p.p.m. naphthylacetic acid had been added. Additional moisture required was supplied as water. The photograph, taken after 19 days of this treatment, indicates tremendous stimulation at 1/100 p.p.m. and, at this stage, substantial stimulation at 1 p.p.m. Subsequently it became evident that the latter treatment was excessive and injurious. Fig. 7 shows the same control pot beside a pot representative of a lot that had received 50 cc. daily of Hoagland's solution plus 1/20 p.p.m. indolylacetic acid, which also stimulated the plants greatly.

Tomato plants were grown in ordinarily good soil for four weeks, then transplanted to exceptionally rich, heavily manured soil, and treated for 17 days. Representatives of groups grown singly in four-inch pots, shown in Plate II, Fig. 8, illustrate two important points. First, the reaction to added hormones is less than in sand: doubtless the naturally occurring indolylacetic acid is present as a product of decomposition in such soil. Second, the extent of stimulation is greatest in very young plants, falling off as the plants grow older. Similar observations were made in several experiments. Nevertheless, the same general type of physiological curve is nearly always observable. In Plate II, Fig. 8, the optimum probably lies between 1/100 and 1/10 p.p.m. naphthylacetic acid, while 1 p.p.m. (fourth from left) has exceeded the maximum. At a later stage, the plants receiving this concentration showed definite injury, as in the sand cultures.

Nasturtiums, started in soil, transplanted after two weeks to four-inch pots of sand, and treated thereafter as in the foregoing experiments, are shown in Plate II, Fig. 9. This photograph was made after the treatment had continued 23 days. The physiological curve obtained again indicates the optimum between 1/100 and 1/10 p.p.m. naphthylacetic acid, while 2.5 p.p.m. clearly exceeds the maximum tolerance of the plants.

Salvias planted in soil in the usual way, then transplanted to four-inch pots, of soil in this case, were then given one treatment, 100 cc. of naphthylacetic acid solution being added to each pot, the concentration adjusted to give dosages of 10, 5, 2.5, 1.25 and 0.1 mg. of the hormone per pot. Eight weeks later, representatives of the usual groups of five pots were photographed. In Plate II, Fig. 10 the control plant, which received only water, is shown at the left. If it were transposed to its logical position at the right, the usual physiologic curve of response would be evident. At this stage, the dosage of 5 mg. appeared optimum, but five weeks later (Plate II, Fig. 11) the next lower dosage of 2.5 mg. appeared fully equal if not superior to it. The lowest dosage, 0.1 mg. per pot, gave well-proportioned plants, heavier in foliage than the controls, with a deeper green color suggestive of a greater chlorophyll content. The heaviest dosage, while stunting the plants somewhat, induced earlier flowering. Most of these differences are observable in both photographs.

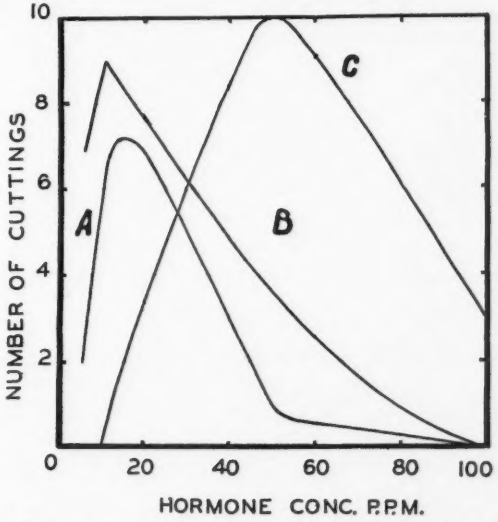
Similar experiments with salvias and petunias, but with the hormone in a dust carrier incorporated in the soil, gave the same general type of results, though less sharply defined.

Lettuce seedlings, growing in soil, were subjected to a more complex experiment, in which the dosages were applied at various concentrations and intervals over progressive periods of time. Plate II, Fig. 12 shows a control flat (left) beside a flat representing approximately optimum effect, this being achieved by 11 treatments at three-day intervals, of 50 cc. 1/100 p.p.m. naphthylacetic acid solution, applied as a fine spray. Solution adhering to the tops was washed down with a water spray. The total amount of hormone applied corresponds to about 120 mg. per acre.

Tomato seedlings were grown and treated similarly. In this case the flats used to illustrate the results (Plate II, Fig. 13) received daily applications of 50 cc. naphthylacetic acid solution, beginning four days after planting. The treatment had continued 16 days when this photograph was taken. The most dilute concentration, 1/100 p.p.m., accumulating a total dosage corresponding to about 200 mg. per acre, gave best results. Solutions of 1 and 5 p.p.m. caused damge, which was very pronounced at 5 p.p.m. Comparing Fig. 13 with Fig. 8, (Plate II), brings out the point already mentioned, that very young plants react more sharply to hormones than do older plants.

Propagation of Cuttings

Experiments with some 4,000 cuttings, representing seven species, have shown dusting with hormones to be a very convenient and successful method of treatment to stimulate rooting. The lower ends of the cuttings, in bunches up to 50 or so at a time, are dipped in the dust, the excess is shaken off, and the cuttings are planted directly. In treatments of this kind the concentration



TEXT-FIG. 1. *Rooting response of solution-treated cuttings, using 5, 10, 25, 50 and 100 p.p.m. A: Salix pentandra with indolylacetic acid. B: Same with naphthylacetic acid. C: Weigela rosea with indolylbutyric acid.*

of the hormone is expressed in parts per million of the dust carrier. An example of the results is given in Plate II, Fig. 14, which shows four specimen groups of *Deutzia crenata* after 26 days in sand in a propagating frame, the three beginning at the left having been dusted at planting with indolylbutyric, indolylacetic, and naphthylacetic acids respectively, in a concentration of 1000 p.p.m. talc dust. The effectiveness of the treatments increases in the same order, the vigor of the naphthylacetic-treated cuttings being especially good. The controls, of which specimens are shown at the right, had not rooted.

Comparisons made with the solution method, already introduced into practice, gave results which were frequently, though not always, in favor of the dusting method. Examples of results obtained by the solution method are shown in Text-fig. 1. Ten cuttings were treated at each of five concentrations, the bases being soaked in the solutions for 22 hours before planting. The curves show the number which rooted in each case. It should be noted that the *Salix pentandra* cuttings (Curves A and B) were dug up after 27 days in sand in the propagating frame, whereas the *Weigela rosea* cuttings (Curve C) were allowed to remain 55 days. The curves indicate a narrower range of tolerance in the solution method than was found in the dusting method.

Responses of Lower Plant Forms

The uniformly positive results obtained with all higher plants tried and the casual observation of similar effects on certain algae, not unlike those found experimentally with algae and just reported by Brannon (1), suggested their applicability to lower forms of plant life. A preliminary series of experiments was carried out on the fermentation of sugar solutions by bakers' yeast. An example of the results is shown in Plate II, Fig. 15, a photograph taken an hour after inoculation, the tubes having stood at a temperature of about 25° C. Gas production was greatly accelerated by 1 p.p.m. naphthylacetic acid (left) and to a lesser extent by 5 p.p.m. (centre) as compared with the control (right). In other experiments 1/10 p.p.m. gave considerable stimulation, and as little as 1/250 p.p.m. had some effect. Higher concentrations than those mentioned have a repressive action.

Similar responses have been obtained with indolylacetic acid, its propionic and butyric homologues, and with the potassium salt of naphthylacetic acid.

Discussion

The hormone treatment of seeds would seem to have important practical applications. The common view that these growth substances repress root growth does not hold when a suitable method of application is used. Experiments in these laboratories, by Dr. R. Newton and Mr. W. R. Jack, have shown that as little as 1/200 p.p.m. naphthylacetic in solution culture depresses the growth of wheat roots in length, though not in weight. Even soaking the seed in hormone solution before planting may have an inhibitory effect on the roots. On the other hand, the dust method seems to be peculiarly adapted to the convenient regulation of hormone supply in such a way as

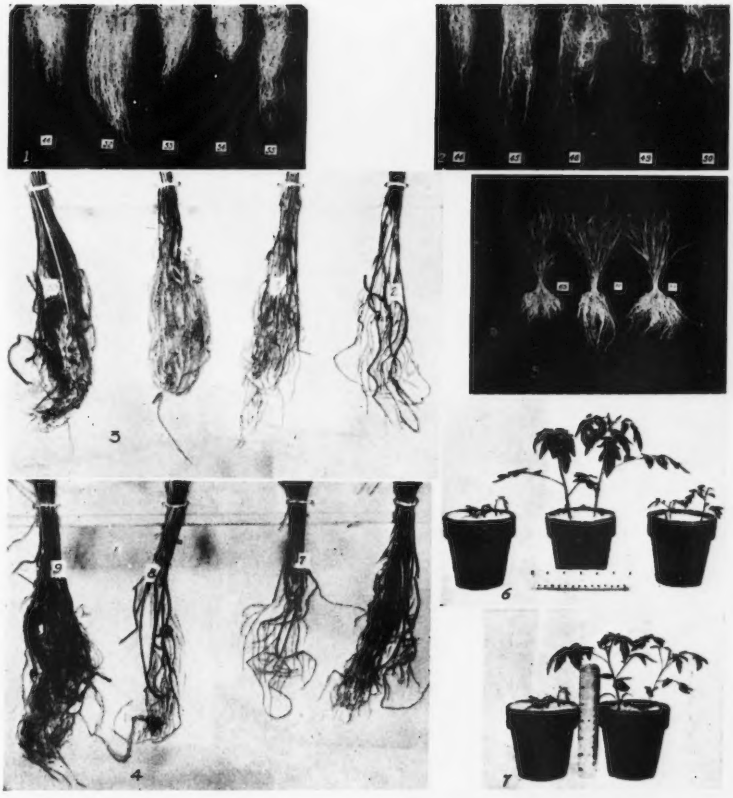


FIG. 1. *Barley roots from dust-treated seed, grown in sand 14 days. Left to right: 0, 2.5, 12.5, 62.5 p.p.m. naphthylacetic acid; and 2.5 p.p.m. each naphthyl- and indolyl-acetic acids.* FIG. 2. *Barley roots from dust-treated seed, grown in sand 14 days. Left to right: 0, 2.5, 25, 50, 125 p.p.m. indolylacetic acid.* FIG. 3. *Soya bean roots from dust-treated seed, grown in soil six weeks. Left to right: 10 p.p.m. naphthylacetic acid; 20 p.p.m., 5 p.p.m indolylacetic acid; control.* FIG. 4. *Soya bean roots from treated seed, grown in soil six weeks Left to right: 10 p.p.m. indolylacetic acid in talc dust; 100 p.p.m., 10 p.p.m. indolylacetic acid in solution; control.* FIG. 5. *Wheat seedlings from dust-treated seed, grown in sand 16 days. Left: Control; centre: 5 p.p.m. naphthylacetic acid; right; 5 p.p.m. potassium salt of same.* FIG. 6. *Tomato plants, 8 days in soil, then 19 days treatment in sand culture. Left: 50 cc. daily nutrient solution only; centre: same, containing 1/100 p.p.m., and right: 1 p.p.m. naphthylacetic acid.* FIG. 7. *Same control plant (left) as in Fig. 6, and (right) 50 cc. daily of 1/20 p.p.m. indolylacetic acid.*

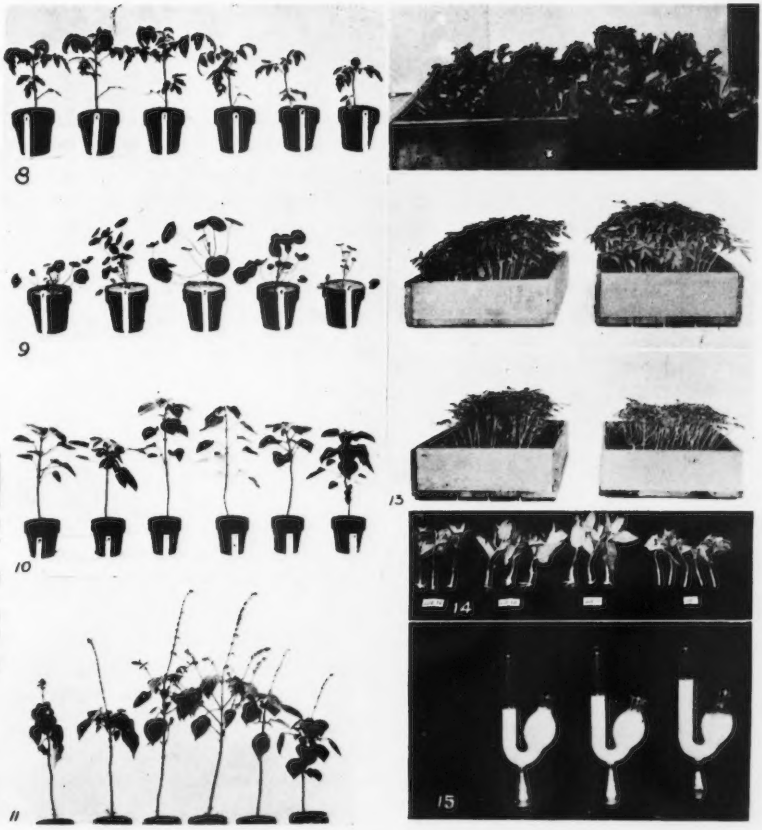


FIG. 8. *Tomato plants grown in soil 45 days, treatment last 17 days. Left to right: 0, 1/100, 1/10, 1, 2.5, 10 p.p.m. naphthylacetic acid.* FIG. 9. *Nasturtiums, 14 days in soil, then 23 days treatment in sand culture. Left to right: 50 cc. daily nutrient solution only; same, containing 1/100, 1/10, 1, 2.5 p.p.m. naphthylacetic acid.* FIG. 10. *Salvias in soil, eight weeks after one treatment. Left to right: 0, 10, 5, 2.5, 1.25, 0.1 mg. naphthylacetic acid per pot.* FIG. 11. *Same plants as in Fig. 10, taken five weeks later.* FIG. 12. *Lettuce in soil. Left: control; right: 11 applications at 3-day intervals, of 50 cc. 1/100 p.p.m. naphthylacetic acid.* FIG. 13. *Tomatoes in soil. Top left: control; right: 1/100 p.p.m. Lower left: 1 p.p.m.; right: 5 p.p.m. naphthylacetic acid, 50 cc. daily for 16 days in all cases.* FIG. 14. *Dust-treated cuttings of* Deutzia crenata, *26 days in sand. Left to right: 1000 p.p.m. indolylbutyric, indolylacetic, naphthylacetic acids; untreated control.* FIG. 15. *Fermentation of 10% sucrose solution by baker's yeast. Left to right: 1 p.p.m., 5 p.p.m., 0, naphthylacetic acid.*

to produce positive stimulation of both root and top growth. Whether the increased early growth so clearly demonstrated in the laboratory and greenhouse can be translated to increased yield of crops in the field, can be demonstrated only by field experiments. Institutions co-operating with this laboratory have included tests with dust-treated winter wheat in this fall's planting. It is hoped that various spring crops can be adequately tested next season.

The field application of "hormonized" fertilizers, or the direct application of hormones to greenhouse or garden crops by spraying or otherwise, also holds interesting practical possibilities. With certain young plants, dosages of the order of 100 mg. per acre have been shown to be effective. However, the optimum dosage will vary with a number of factors, such as method of application, composition of the soil, and meteorological conditions, in addition to the kind and age of plant. There is undoubtedly marked adsorption of these active chemicals by soil. Stimulation is effected by an optimum amount of free chemical and that, of course, can be attributed to an equilibrium of some description at the soil–root interface. Our results suggest that for optimum stimulation the available amount of hormone at this interface must be substantially lower than 1/100 of a part per million, almost certainly very much lower than this value.

Greenfield (4) lately reported optimum stimulation of *Matthiola incana* by indolylacetic acid applied to the soil in seven-inch pots at a rate which works out to about 2000 *grams* per acre. This lies in the range of about 1500 gm. per acre found most effective with the Salvias shown in Figs. 10 and 11 (Plate II). On the other hand, the effective amounts in the experiments with young lettuce and tomato plants illustrated in Figs. 12 and 13 (Plate II) lie in the radically different range of 50 to 250 *milligrams* per acre. Co-operative field experiments to test the practical utility of these effects are planned for next season.

The dust treatment of cuttings has so far indicated a balance of superiority over the solution method, with respect to effectiveness. Even if we assume it to be no better in its results, its simplicity and convenience still recommend it for practice. Further experiments may prove the one treatment more suitable for certain species or conditions, and the other better adapted to certain other cases.

The marked swelling of herbaceous cuttings when the cut end is immersed in hormone solutions not only gives a clue to the possible mechanism of hormone action in the plant: it also suggests other useful applications. Immersing the base of partly wilted lettuce plants and cut flowers in appropriate concentrations causes these to regain their turgor and freshness in a remarkable way. The life of certain cut flowers may thus be prolonged.

The response of lower plant forms to hormones suggests their possible utility in industrial processes and fermentations. This field is being explored by a number of other workers in these laboratories. It is of incidental interest

that the quick response of yeast has already led to its use by the writer in assaying quickly and conveniently the activity of a mixture of 1- and 2-γ-naphthylbutyric acids, prepared by Dr. R. H. Manske in these laboratories.

Acknowledgments

The indolyl compounds were prepared by Dr. R. H. Manske, the naphthylacetic acid by Dr. A. Cambron, both of these laboratories. Grateful acknowledgment is made to them for large supplies of these chemicals in pure form during the past year.

The writer wishes to express his appreciation of the assistance given by Dr. R. Newton, Director of the Division of Biology and Agriculture.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 15, SEC. D. NOVEMBER, 1937 NUMBER 11

THE MALE GENITALIA IN THE HYMENOPTERA (INSECTA), ESPECIALLY THE FAMILY ICHNEUMONIDAE[1]

I. COMPARATIVE MORPHOLOGY

BY OSWALD PECK[2]

Abstract

The trend of specialization in the male genitalia and the adjacent sclerites is traced within the order Hymenoptera (Insecta) and the morphological problems of these parts discussed. In the Ichneumonidae, these sclerites appear to have little or no tribal or subfamily characteristics of practical value to the taxonomist, such differentiation being masked extensively by specific variation. Thirty-four tribes and ninety-six species were studied. One hundred and fifty-eight figures are shown.

Introduction

In the family Ichneumonidae, the determination of species is often extremely difficult. The keys to the family are based partially upon the characters of the female and cannot always be relied upon for the identification of the males.

Furthermore, it is not at all certain that the present systematic division of the family is a natural one, the situation being summarized by Cushman and Rohwer (32), who expressed the belief that: "The family Ichneumonidae is a group composed of elements showing remarkable differences but at the same time extreme homogeneity. So true is the latter that the grouping into five universally recognized subfamilies leaves the placing of a species in its proper subfamily almost entirely to the imagination or experience of the worker. On the other hand, the strict interpretation of such characters as these keys offer frequently leads even the experienced taxonomist to entirely misplace an insect; and disagreement among workers as to the allegiance of certain genera is very frequent."

This view was supported by Viereck (135) who keyed out the ichneumonid species without defining either the traditional subfamilies or the tribes. The difficulty in defining the higher groups is shown also by Schmiedeknecht (116), who recognized 13 tribes in the Pimplinae, yet keyed out the genera in the family without direct reference to the tribal grouping.

[1] *Manuscript received October 26, 1937.*
Joint contribution from the Farnham House Laboratory, Imperial Institute of Entomology, England; the Department of Entomology, McGill University, Montreal, Canada, and the Division of Systematic Entomology, Entomological Branch, Department of Agriculture, Ottawa, Canada. Largely an abstract from a thesis approved for the degree of Ph.D. from McGill University, October, 1936.

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Under these circumstances, it seemed possible that a thorough study of the male genitalia might help to solve these difficulties.

This viewpoint was supported by the presence of tribal characteristics in the ovipositor of the Pimplinae (32), as well as by the constant elongation of the male claspers peculiar to the *Mesochorini*. Furthermore, while specific characters had been shown in the male genitalia of many other hymenopterous groups, this, in itself, did not preclude the presence of tribal ones as well.

In their structural characters, the members of the family Ichneumonidae are regarded as being intermediate between the Chalastogastra (sawflies and horntails) and the aculeate Hymenoptera (ants, bees and wasps). A study of the comparative morphology of the male genitalia in these forms should thus be of value since it may (1) allow us to recognize and define the different stages of morphological specialization existing within the Order, (2) provide data which will assist in making a more rational arrangement of the larger groups within the Order and the families, and (3) aid in the establishment of a consistent, morphologically sound system of nomenclature within the Order.

The term *genitalia* is usually applied to the organs of copulation, which are morphologically external, although it is sometimes taken as including the internal reproductive organs. Studies of the latter are already available in the Ichneumonidae (13–16, 20, 79) and will not be considered here. However, as the specialization of the hymenopterous genitalia in the male usually involves modifications in the remainder of the genital segment, and these, in turn, affect the tenth and eleventh abdominal segments, the study of the genital region was extended to include these segments also, when necessary.

Comparative Morphology of the Male Genitalia

A. The Male Genitalia in the Hymenoptera

1. *Introduction and Definitions*

An extensive but superficial study of the male genitalia of the Hymenoptera was made by Leon Dufour (34). Supplementary efforts by later workers produced morphological chaos by their failure to give the same names to the same parts. Boulangé (17), by the publication of tables indicating the terms used by the various workers, introduced a semblance of order; however, this author, like Richards (96), preferred to use noncommittal terms, having no precise morphological significance outside of the order, this procedure apparently being based upon the belief, expressed by Richards, that "it is not possible in my opinion to homologise with certainty the parts of the hymenopterous genitalia with those of less specialized orders".

Snodgrass asserted (122, 123) that the parts of the male genitalia in the endopterygote insects can be identified by their musculature and articulatory relationships, although later (124) he described the genitalia "regardless of what may be the morphological relations of the latter". It is certain that in the initial stages of morphological work a non-significant or merely anatomical system of nomenclature is a convenience and, indeed, a necessity; nevertheless,

the establishment of a uniform system of nomenclature, based upon morphological homologies, throughout the whole range of the group studied, is, as all agree, the ultimate objective of morphological investigation.

It seems evident that, if homologization is possible between the ichneumonid genitalia and those of other hymenopterous and endopterygote groups, it must be based upon the primitive sub-order Chalastogastra, its parasitoidal family, the Oryssidae, perhaps being intermediate. Boulangé (17), studied the genitalia and their musculature in the Chalastogastra, in *Vespa* and in *Bombus*. He arranged the various structures in series, the members of each group clearly corresponding in position to one another. However, using myology as the criterion, he regarded the inner clasper of *Bombus*, but not of *Vespa*, as homologous with the volsella (or inner clasper) of the Chalastogastra, even though some similarity in form and function existed in *Vespa*.

As will be shown later in this paper, the parts of the male genitalia in the Ichneumonidae are clearly homologous with those in the Chalastogastra and therefore, at least, with those in the Apoidea also.

The male genitalia of the parasitoidal Hymenoptera, so far, have received very little attention, though the genitalia of some chalcids were described by Embleton (36), Imms (56), Grandi (44–46), James (57) and Hanna (51); the development of these appendages in the braconid *Doryctes* was observed by Seurat (118) and their structure in a few species of ichneumonids and braconids was studied by other workers (8, 21, 26, 43, 82, 109).

An effort has therefore been made in the present paper to identify the parts of the male genitalia of the ichneumonids with those of the Chalastogastra and with the more generalized forms of insects. Until this is accomplished, it is obviously impossible to place the terminology upon a sound basis.

As the hymenopterous genitalia have a series of sclerites peculiar to this order, a system of names must be selected for these parts, the choice being according to the claims of priority or custom, preferably the former.

The nomenclatorial systems of the various workers were tabulated by Boulangé (17). The earliest of these were proposed by Audouin (6), Hartig (52), Newport (75), Dufour (34) and Schenck (113). Unfortunately, these workers did not confine themselves to the use of similar terms for parts common to the main groups. The application of the law of priority would result in creating a legal monstrosity derived from several systems and thereby losing most of its value; furthermore Audouin's term *spatha* would have to be applied in a sense that he alone recognized, although the term is widely used for the dorsal portion of the intromittent organ. Under these circumstances practicability is the logical criterion.

The next system in order of priority is that of Thomson (127) and there seems to be little to choose between it and that of Hartig. The latter system was sponsored by Rohwer (101) but otherwise was ignored. The former is followed by Mickel (64, 65), Richards (94-96), Ross (108) and Ries (97) who

are some of the chief workers in this field at the present time. Thomson's terms were more widely used than those of any other author and are applicable throughout the order, except, perhaps, with regard to the inner clasper of vespids. They were drawn from the supposed homology or analogy between the gnathal and genital appendages and, to differentiate between them, Crampton (22) suggested that the prefix "gono-" be used for the latter series. To be consistent, it is necessary also to change the term squama to *gonosquama* to prevent confusion with the dipterous squama.

However, Thomson's terms were originally applied to bombids, in which the outer clasper is divided transversely into gonostipes and gonosquama. In the ichneumonids, these are usually fused into a compound structure for which there is no entirely satisfactory term, as the terms gonopod, coxopodite, stylus, harpe and harpago are all morphologically incorrect, while the term stipes-squama of Richards (96) is clumsy, particularly if the prefix gono- is added. The term *forceps* was chosen in 1841 by Dufour (34) but the same term was previously applied to the dermapterous cerci by Burmeister (18). As the word then is not applicable to the outer claspers in the Hymenoptera, the writer proposes the term *gonoforceps* for the latter structure when the gonostipes and gonosquama are fused together.

Since the word *sagitta* was first used for the penis valve in *Bombus* (127), and since this structure is homologous with the paramere, the term should be sunk as a synonym. However, recent writers have used the term for various sclerites, especially for the articulatory sclerite at the apex of the inner clasper; Thomson named this part the lacinia so that, for the sake of consistency, we should now term it the *gonolacinia*.

Other morphological terms used in this paper conform to the usage of Thomson (127), Boulangé (17) and Snodgrass (122-124), excepting the terms aedeagus and paramere, which agree with some concepts of Snodgrass (122, 123) but not his latest (124). Many of the terms, however, are explained in the text. The hypandrium, outer claspers, inner claspers and harpago are considered as denoting function rather than morphological entities.

2. *The Genital and Postgenital Terga*

Among all endopterygote insects, including the Hymenoptera, Trichoptera, Mecoptera, Lepidoptera, Diptera and Coleoptera, the male genital segment is invariably the ninth abdominal. Behind this segment lies the gonopore or genital opening, in the intersegmental membrane and between the two appendages known as the *gonopods* (122, pp. 17, 18).

Among the Hymenoptera, it is but seldom that the tergum of the genital segment is not radically modified. This tergum may undergo a sequence of changes, the various steps, in order, being: (i) invagination, (ii) division into a pair of lateral *genital tergites* (Tg. IX, Figs. 16, 17, 21), (iii) fusion of these genital tergites with the tergum of the tenth segment to form a *syntergum* (Fig. 20) or, alternatively, fusion of the genital tergites with the tergites of

the tenth segment, forming a pair of *syntergites* (Sntt., Figs. 5, 6, 22), and (iv) a reduction in size and functional importance of the syntergites, accompanied often by the loss of the pygopods (Figs. 18, 19).

When the tenth tergum is fused with the ninth, the former may be distinguished by (i) the intimate attachment of the rectum by muscles to the posterior portion of the syntergum, (ii) the points of attachments of the inter- and intra-segmental muscles in comparison with those of the pregenital segments and (iii) the ventro-lateral proximity of the *pygopods* (commonly termed cerci), when these appendages are present. The genital tergites in most ichneumonids have a distinct antecosta; this is absent in the tenth tergum.

In the Chalastogastra, the most primitive group of Hymenoptera in existence, the ninth tergum is usually divided into two lateral tergites. This is true of most siricids (22), although in *Sirex juvencus* L. these tergites are joined by a narrow, sclerotized bridge (17). It is true also of *Xiphidria mellipes* Say (22) and of some species of *Cephus* (17). The incomplete and complete separations of the tergites are shown in *Cephus cinctus* Nort. and *Pteronidea ribesii* Scop. respectively (Tg. IX, Figs. 15, 16).

In contrast to the great majority of the Chalastogastra, the parasitoidal oryssids have the male genitalia completely invaginated (38). In *Oryssus sayi* Westw., as probably in the other members of this sub-order, this invagination has been accompanied by the ninth and tenth terga being reduced in size and in sclerotization, the ninth tergum being divided also into lateral tergites. In this reduction in size, the Oryssidae are more specialized than either the Chalastogastra or the Ichneumonidae. Rohwer (98) noted that cerci (*i.e.*, pygopods) were absent in the Oryssidae; however, in *O. sayi* they are merely concealed by the invagination.

The ichneumonid genital tergum is always divided medianly and may form (i) a pair of tergites (Tg. IX), as in *Pimpla coelebs* Walsh (Fig. 21); this is rare within the family; (ii) a syntergum (Snt), as in *Exeristes roborator* Fabr. (Fig. 20); or (iii) a pair of syntergites, as in *Megarhyssa lunator* L. (Fig. 6) and *Banchus falcatorius* Fabr. (Fig. 22, Sntt.). Intermediate forms also occur.

The chalcids appear to possess a syntergum and a rudimentary eleventh tergum, the syntergum being in many cases identifiable by the possession of pygopods (44-46, 51, 57). It must however be noted that in the figures of these authors the pygopods show that their "ninth tergum" is a syntergum and that Grandi (44-46) incorrectly identified the gonocardo as the tenth segment. The primitive chalcid, *Brachymeria intermedia* Nees, is primitive in this respect also, for the ninth and tenth terga are separate, although the former is medianly divided (Fig. 17).

In the aculeate Hymenoptera, the ninth and tenth terga appear to be always fused (96) and this fusion is usually accompanied by extensive invagination and the loss of the pygopods. In the Chrysididae, only four to six

abdominal terga are visible externally (19). Although both Wheeler (137) and Donisthorpe (33) hold that there are ten distinct abdominal segments in the Formicidae, yet the ant *Lasius niger* L. has the sclerotic area of the syntergites almost entirely reduced, although their identity is shown by the prominent pygopods (Fig. 18, Pyg.). Syntergites (Sntt.) are present also in *Vespa maculata* L. (Fig. 19), *V. germanica* F. (17), *Bombus terrestris* L. (17), *Colletes cunicularius* L. (72), *Andrena wilkella* (Kby.) Ill. (Fig. 14), *Halictus lerouxii* Latr. (Fig. 13) and in *Apis mellifica* L. (120). This agrees with the contention of Richards (96) that, in the aculeate Hymenoptera, the ninth tergum is apparently always reduced to a pair of small syntergites.

While it is evident that the ninth tergum, when fused to the sclerite or sclerites posterior to it, is indubitably in direct contact with the tenth tergum, yet the morphological significance of the latter sclerite is not entirely plain. As noted above, the pygopods are borne upon the tenth segment. According to Boulangé (17, p. 218), they are appendages of the tergum, although Middleton (66) has rightly claimed that in *Pteronidea ribesii* they lie ventrally to this sclerite. However, the series of chalastogastrous and ichneumonid adults examined during this study suggest that the pygopods lie immediately postero-ventrally to the tergum, arising in the intersegmental membrane. Snodgrass (122, 124) holds the view that they are not cerci (*i.e.*, not appendages of the eleventh segment) but may be homologous with the socii of the Lepidoptera. Whatever their morphological significance may be, these appendages serve as admirable landmarks of the postgenital segments.

The eleventh tergum varies considerably, both in the degree of sclerotization and in its relationship to the tenth tergum. Berlese (11) claimed that the tenth and eleventh terga were fused together in *Cimbex americanus* L. and separate in *C. axillaris* Pz. This inter-tergal fusion was believed by Crampton to probably form the chalastogastrous epiproct (22). Boulangé (17) found in *Xeris spectrum* L. (Siricidae) and *Cephus pygmaeus* L. (Cephidae) evidence of fusion through the persistence, in an attenuated state, of the musculature of the two postgenital terga. The same author believed that a similar fusion occurred in *Bombus* and *Vespa*.

However, Snodgrass (122, p. 97; 124, pp. 253, 605) considered that the postgenital dorsal sclerite in the Hymenoptera is that of the tenth alone. This is supported by the existence of a broad membranous area behind the last sclerite in many chalastogastrous and ichneumonid species. In the sawflies *Pteronidea ribesii* and *Dolerus unicolor* Beauvois this sclerite bears strong setae and setal alveoli respectively, the setae being similar to those of the tenth tergum; in these cases, the setal remains surely must be landmarks of a primarily sclerotic area, which can only be the eleventh tergum. This identification is also supported by the invariable occurrence of the ichneumonid pygopods in the membrane immediately behind the last functional tergum, suggesting that the eleventh tergum is invariably either fused to the tenth or else de-sclerotized, at least in the ichneumonids.

The further exploration of this problem is outside the scope of this paper, but the morphological value of the pygopods as landmarks makes some reference to it essential, in order to show the significance of the post-genital terga and therefore of the syntergum and the syntergites.

3. *The Genital and Postgenital Sterna*

In the Hymenoptera the ninth sternum may be specialized by (i) invagination, the ninth sternum lying dorsally to the eighth, (ii) reduction in sclerotization and (iii) fusion with the eighth sternum. The hymenopterous *hypandrium* is usually formed of the ninth sternum alone, but in at least some of the Vespidae, the eighth sternum is fused with the ninth. The composite character of the hypandrium in the latter case is usually indicated by the presence of (i) the median *spiculum*; (ii) the *antecostae* of the two sterna and (iii) the inter-segmental and intra-segmental muscles.

The ninth sternum is well developed in the lower Hymenoptera, such as the Chalastogastra (17), the Idiogastra and the ichneumonids, as well as chalcids (44-46, 51, 57). The aculeate hypandrium is reduced in size and lies dorsally to the eighth (17, 70-72, 110-112). Atwood (5) regarded the eighth and ninth sterna of *Halictus* and *Andrena* as formed by a secondary division of the eighth, the gonocardo being the "base of the ninth ventral segment". In *Halictus lerouxii* and *Andrena wilkella*, a strong muscle (Boulangé's *muscle en sangle*) extends from the anterior part of the syntergite (*i.e.*, from the genital tergite) to the ninth sternum and short inter-sternal muscles exist between the eighth and ninth sterna. In *Andrena wilkella* the acrosternites (Ast.) and antecostae (Ac.) of the two sterna are plain (Fig. 14). Furthermore, in this species, the spiculum (Sp.) has been bent posteriorly through invagination, forming an internal median ridge upon the ninth sternum. In Atwood's figures, the antecosta and spiculum of the ninth sternum are plainly shown as darkly stippled areas. In *Apis mellifica* the ninth sternum is "a well-developed semicircular band, forming the ventral and ventro-lateral parts of the ninth segment. It bears on each side, two conspicuous lobes" (Snodgrass (120)). These lobes are the claspers.

Among the wasps, specialization has been carried further by the fusion of the eighth and ninth sterna (17, 58, 132, 138). The vespid hypandrium has been incorrectly interpreted as the eighth sternum and the gonocardo as the ninth by Balfour-Brown (7). The hypandrium of *Vespa* is composed of two sterna (17), while the two antecostae, spiculum, and segmental muscles are also prominent (Figs. 36, 37).

In contrast with the development and subsequent degeneration of the ninth sternum, the tenth and eleventh are always retrograde structures, usually being indefinitely demarcated and semi-membranous. This is plainly due to their early invagination between the terga and the gonopods.

In some ichneumonids, notably *Pimpla instigator* Fabr. and *Megarhyssa lunator* Fabr., the rigidity of the post-genital sterna is still well preserved. All ichneumonids have the ventral margin of the anus and the adjoining

rectum supported by a sclerotic area; the latter is usually divided medianly and varies in shape from hyperbolic to V-shaped and quadrate. In *Ephialtes tuberculatus* Auct., *nec* Fourcr., these sclerites bear strong setae with large alveoli, suggesting strongly that these anal structures are not secondarily developed but are remnants of the eleventh sternum, particularly as they lie ventrally to the membranous area that seems to be the eleventh tergum.

In the chalcid, *Brachymeria intermedia*, the post-genital sterna appear to have fused, forming a long rod that joins the lower lip of the anus to the base of the genitalia (Fig. 38); its fusion is not clear and no modification of this kind has been described in other chalcids by Grandi (44-46), James (57) or Hanna (51).

As the anal sterna are fragile and minute, as well as being only indirectly concerned with genitalia, they have not been included in this study; however, they appear to have acquired their present form early in the evolution of the Hymenoptera and may possess group characteristics of interest.

4. *The Genital Appendages*

Since the basal, annular gonocardo is derived from the gonopods, it is an integral part of the male genitalia in the Hymenoptera, although in recent years it has been misinterpreted as the ninth segment by Grandi (44-46), Balfour-Browne (7), Atwood (5) and Abbott (2).

(a) The Typical Structure. The male genitalia differ considerably in the various families of the Hymenoptera, yet all exhibit the same fundamental structure, as shown by Boulangé (17) in the Chalastogastra (Figs. 9-11). Except in the honey bee, four main parts are distinguishable in the adult, these being (i) the basal annular *gonocardo* (Gc.), which bears latero-posteriorly (ii) a pair of hollow, hemi-ellipsoidal outer claspers or *gonoforcipes* (Gf.), each of which supports by its antero-ventral margin (iii) the inner clasper or *volsella*. The gonoforcipes and volsellae support, by means of muscles and membrane, a median intromittent organ, later shown to be (iv) an *aedeagus* (Aed.).

The gonocardo is attached to the intersegmental membrane lying posteriorly to the ninth segment; it sometimes bears a medio-ventral apophysis, the *gonocondyle* (17). Dorsally to the gonocondyle or gonocondylar area lies the tip of the *gonostipital arm*, an anterior elongation of the *gonostipes* or basal portion of the gonoforceps. The apical part of the gonoforceps is frequently differentiated into a distinct appendage, the gonosquama, which may be articulated. Gonosquamae are present in most Chalastogastra (22), in a few ichneumonids and vespids, in bombids and in other aculeate Hymenoptera; in the ichneumonids they are not articulated.

The gonostipes bears the volsella, which, among the lower families, normally lies in the vertical plane. The volsella often assumes bizarre shapes. It frequently bears an apical articulatory sclerite, designated in this paper the *gonolacinia*, although termed the *pièce en trébuchet* by Boulangé (17), the *middle clasper* by Peacock (80) and the *squama* by Salt (109) and Glover (43). The word squama was originally applied by Thomson (127) to the apical portion of the outer clasper in *Bombus*.

The aedeagus has no close articulation with the remainder of the genital sclerites. In its unspecialized form this organ consists of a pair of lateral *parameres*, sometimes termed the *sagittae*, joined both dorsally and ventrally by membrane which may become secondarily sclerotized. The dorsal portion of this membrane may be distinguished as a heavily sclerotized area and then is termed a *spatha*. Each of its antero-lateral corners forms a lateral apophysis, Boulangé's *ergot*, which forms a fulcrum about which the aedeagus can pivot in the sagittal plane; the ergot is usually strengthened by accessory *spathal rods*. Anteriorly the parameres extend far into the body cavity. The aedeagus may assume remarkable modifications in form and size, as in *Bombus*. It is usually large in the chalcids (34, 36, 44-46, 51, 56, 57), dwarfing the small outer and inner claspers. In the hive bee also the aedeagus is abnormally large but, being eversible, it is mainly membranous (120).

(b) Ontogenetic Development. In all Hymenoptera the histoblasts of the male gonopods are situated in the twelfth larval segment behind the head (*i.e.*, the ninth abdominal segment of the adult). This has been demonstrated in the Chalastogastra (17), Proctotrupidae (35), Ichneumonidae (119, 126), Braconidae (42, 76, 131), in the Vespidae, Bombidae and Apidae (138) and in other groups.

A study of the ontogenetic development in *Sirex* (17), in the braconid *Doryctes* (118) and in *Vespa* and *Bombus* (138) shows that each histoblast develops into a primary papilla that divides longitudinally into the outer and inner claspers. A transverse basal sclerite is separated from the base of each primary papilla, fusing with its homologue from the other gonopod to form the annular gonocardo.

According to Zander (138-140) the parameral papilla in Lepidoptera, Trichoptera and Hymenoptera is formed from the base of each primary papilla, soon to fuse with its fellow and with the ejaculatory duct to form an aedeagus. This mode of the formation of the parameres was generally accepted as true until recently, when Mehta (62) claimed that the parameres in Lepidoptera develop earlier than the lobes of the gonopod and independently of the primary papilla. Moreover, Mehta was able to support his ideas by citing evidence from the Hymenoptera (63) and from the other main orders in the Endopterygota. The significance of this is discussed in the next section.

Among the Hymenoptera the formation of the genitalia is always along somewhat similar lines, but variations occur, both in the number of adult appendages and in the order of their differentiation. Unfortunately, most workers upon this aspect studied the genitalia of the honey bee, in which these structures are aberrant: only two pairs of secondary papillae have been found in this insect so either the parameres are absent (120, 138) or else the outer and inner claspers remain undifferentiated (17, 63).

Of greater interest is the disagreement between the developmental and myological evidence. The chronological development in *Sirex*, *Vespa* and *Bombus* is shown diagramatically (Fig. 1); unfortunately, the description of the braconid *Doryctes* by Seurat (118) is inadequate for this purpose. The

differentiation of the parameres (Pr.) occurs later than the separation of the individual outer and inner claspers (O.C. and I.C.), both in *Sirex* (17) and *Vespa* (138), although their volsellar muscles are not homologous (17). Yet the reverse is true of *Bombus*, for the parameres are differentiated before the appearance of the two pairs of claspers (138), although the genital muscles are homologous with those of *Sirex* (17).

The discrepancies between these data suggest that the ontogenetic evidence should be interpreted cautiously but suggest the independence of the parameres from the gonopod and thereby provide support for the contention of Mehta (62) that the claspers but not the parameres are gonopodal in origin.

Before our available ontogenetic data can be of value in studies of the hymenopterous genitalia, they must be adequately verified and supplemented. At present they appear to be of little use in comparing the genitalia of adults, unless supported by other evidence.

(c) The Morphological Significance. According to Snodgrass (122, 123), the typical male gonopod of an adult insect consists fundamentally of a basal *coxopodite* and an apical stylus or *telopodite*, the coxopodite bearing an unsegmented *paramere* on its inner margin. The coxopodite may be free, fused to its fellow, or joined to other parts of the genital segment. The parameres of the higher insects are fused to the terminal portion of the ejaculatory duct, forming the median intromittent organ, while the stylus is represented by the clasper; each of the latter may be divided into a pair of claspers also (122, p. 192). Accessory structures may be present but almost invariably they have no muscles and are therefore distinguishable from the paramere and stylus, which are attached by muscles to the coxopodite.

It must be remembered that the origin and position of appendages are not the sole guides to their homologies, for the direction of articulation of limbs is an unusually stable landmark (122). Since the gonopods are believed to be homodynamous with the thoracic limbs, a comparison of the genital claspers with the primitive thoracic limb may be of value in determining the homologies of the genitalia.

The primitive thoracic limb consists of the basal coxopodite (Cxp.) and the distal telopodite (Tlp.) with muscles extending from the base of the coxopodite to the base of the telopodite, as in Fig. 2 (121, 124). The coxopodite moves in a horizontal plane upon the body, the secondarily differentiated coxa moving in the same plane also, while the telopodite articulates vertically upon the coxopodite (121, 124), as shown in Fig. 4. Since both the primary papillae of the thoracic limbs and the genital histoblasts are formed upon the ventral surface of the body, the initial inherent movement of the undifferentiated coxopodite is sagittal. The subsequent development of the insect causes the thoracic papillae to migrate laterally, while the genital appendages move posteriorly; the coxopodite of the latter therefore secondarily articulates in a vertical plane, while the telopodite moves horizontally (121). The significance of this is shown later.

The term paramere was proposed by Verhoeff (133) for a paired appendage lying laterally at the base of the coleopterous penis. Since this time, this term has been applied loosely, especially when the parameres are united as an aedeagus. The location of the coleopterous parameres upon the penis shows that these structures are phallic, not gonopodal, and with this Snodgrass (124) concurs. Yet the parameres of the endopterygote orders are widely believed to be gonopodal in origin, as stated by Snodgrass (122, 123). Snodgrass' conclusions upon the origin of the parameres may also have to be modified if the above-mentioned ontogenetic findings of Mehta (62), prove to be valid. Substantiated by the data of workers in other orders, Mehta's work indicated the probability that the basic data, as outlined by Snodgrass (122, 123), are partially incorrect, the parameres never being gonopodal. Should this be so, Mehta has clarified considerably the current concepts, not only upon parameres, but also upon the fundamental structure of the male genitalia in the Endopterygota, a group in which a common basic form is to be expected.

Recently Snodgrass (124) has reviewed the male genitalia of pterygote insects and his conclusions are quite at variance with his previous ones (122, 123). The term paramere is accepted in the coleopterous sense but in this work he has adopted a terminology "that can be applied consistently to the major structural elements *regardless of what may be the morphological relations of the latter*" (the italics are mine). The genital structures are divided into two classes, phallic and periphallic. The phallic organs are "immediately concerned with the function of coition; they include the phallus and various accessory or supporting structures associated with the latter. The periphallic organs are movable or immovable lobes or processes that have for the most part a grasping or clasping role in the function of copulation" (p. 586). The phallic organs, including the parameres, are evolved from the conjunctival membrane posterior to the ninth sternum or in the genital chamber anterior to the membrane. The "periphallic structures . . . . arise peripherally, generally from the annulus of the ninth abdominal segment but also from the other segments often closely associated with the latter in the genital complex" (p. 591). They may include a pair of lateral movable claspers and various immovable lobes or processes arising from the tergum or sternum; the movable claspers or *harpagones*. To me, these concepts seem inadequate for the positive identification of some of the genital appendages.

Applying these concepts to the Hymenoptera, Snodgrass suggests that their genitalia are entirely phallic, the gonocardo and gonostipites being formed from the phallobase, while the median appendage is an aedeagus; the gonosquamae "may be termed parameres since they are at least analogous with the parameres of Coleoptera", even though he states that the apices of the parapenes are "structures of the same nature" as the gonosquamae. How little is realized of the morphology of these hymenopterous structures is shown by these suggestions that the parameres may be either the gonosquamae or parapenes, or, if the word aedeagus is to be construed rigidly, even a part of this median organ also.

Snodgrass' conclusions are not entirely consistent with his assumptions, the claspers in the Hymenoptera being considered as radically different from those of allied orders. The claspers in the Lepidoptera arise from the genital cavity (62, 140), as do those of the Hymenoptera (63, 138); yet the former are termed periphallic and the latter phallic, even though their functions are similar. Furthermore, the derivations of the main genital parts in the Lepidoptera, Trichoptera and Hymenoptera are at least similar enough to permit their homologization by Zander (138-140). Even the muscles at the base of the clasper in *Panorpa germanica* L. suggest the affinity of this structure with that of the Hymenoptera (17); yet the mecopterous appendages are termed periphallic. Because of these data, the hymenopterous clasper seems to be homologous with those of allied orders.

The parameres and other genital appendages have been identified also by Pruthi (85). This author considered the parameres to be derived from the median papillae and distinct from the gonopods, which are in the same general plane as the ninth sternum, lying under the penis and parameres. Thus Pruthi agrees with Mehta (62) that the parameres are attached to the base of the penis (it should be noted that Pruthi has interpreted the penis as the aedeagus).

From the ontogenetic data it is plain that the hymenopterous gonopod is basally fused to its fellow, forming the gonocardo (142). This fusion is indicated in the adult by a medio-ventral or medio-dorsal suture or else a corresponding internal ridge; the ventral fusion is shown in many species of ichneumonids, while the dorsal fusion is plainly shown in *Nototrachys foliator* Fabr. and *Trogus lutorius* Fabr. The gonocardo therefore must represent either the two coxopodites or else their bases.

As the median intromittent organ is derived from the fusion of the ejaculatory duct with paired rudiments, this composite organ is evidently an aedeagus. The Chalastogastra, a primitive group in the order, has a primitive aedeagus also, for it often consists almost entirely of the duct, of the two elongate sagittae and of connective membrane; it seems evident that the parameres are represented by the sagittae, especially since the latter are found throughout the members of the order (except possibly *Apis mellifica*) and form the inflexible lateral parts of the compound organ.

The outer and inner claspers are provided with well developed muscles, so that according to Snodgrass (122) neither can be purely accessory structures. Crampton (23, 24) suggested that the gonosquama is the stylus and Richards (96) supported him by indicating that the gonostipes and gonosquama probably form the apical portion of the coxopodite with the stylus. The latter author suggested also that the volsella is a two-segmented paramere and the median organ an aedeagus; his basic definitions evidently differ from those of Snodgrass (122, 123). Becker (8) concluded that the volsella of the Tenthredinidae and Ichneumonidae represents the trochanter and femur, and the bombid volsella the femur. The gonosquama then presumably would

be an epipodite. As Becker appears to have compared the hymenopterous genitalia directly with those of the Apterygota, his failure to study intermediate forms seems to invalidate his conclusions entirely.

A homology between the volsella and the telopodite is suggested by the constant presence of the articulating gonolacinia in the lower families of the Hymenoptera. Moreover, the volsella in the Chalastogastra and Ichneumonidae has a constant, diagonal, internal thickening, the *volsellar strut* (V. Str.) (Figs. 9, 117–127). This suggests the remnant of the arthrodial thickening in a flattened limb joint, the adjacent limbs being joined by Boulangé's muscle Q (Fig. 8). However, this strut appears to be secondary, for its function is to prevent buckling of the volsella during the contraction of Boulangé's muscle Q. Furthermore, there are no condyles in the gonostipito-volsellar joint, although they should be present in an articulatory limb that is derived from a primitive leg.

The lack of homology between the volsella and the telopodite is further shown by the base of the volsella being almost flat in the primitive Hymenoptera, so that, if this homology were correct, the telopodite must have changed basally from the normal tubular form to one that has been either very strongly compressed or else split along the main axis of the limb, spread outwards and flattened.

The first method of evolution is impossible, for there is no sign of fusion between the opposite sides of the limb (except possibly the volsellar strut), the basivolsella being thin and almost diaphanous. Furthermore, the intrinsic muscles of the tubular limb must have been gradually replaced by (or developed into) an efficient and complex extrinsic system. It is absurd to postulate the occurrence of both of these radical changes, each of which is highly improbable.

The second method of formation seems to be equally impossible. The longitudinal fission and unfolding of the basal part of the telopodite, accompanied by extensive internal changes, could only have evolved slowly, yet intermediate forms appear to be absent. Furthermore, this change from the tubular form to that of a plate would weaken the telopodite, probably at the very time that it was being adapted as a clasper, a structure that is essentially strong. This theory can be accepted only if accompanied by further proof, and in the absence of a more logical explanation.

The absence of homology between the telopodite and the volsella is shown by the origin of the latter. This structure is formed from the lateral papilla by an apical or medio-apical fission, the volsellar region being the smaller (Fig. 1). This suggests that the volsella is not the entire telopodite, although possibly an endite.

The fundamental difference between the telopodite and the volsella is shown also by musculature. The base of the paramere is joined to the base of the gonolacinia by Boulangé's muscle N in both the Chalastogastra (Fig. 8) and the Ichneumonidae (Fig. 7). Even if it is conceded that the paramere is gonopodal in origin, one cannot recognize the existence of a primary muscle

that extends between a basal appendage of the coxopodite and the apical telopodite. Either this muscle is secondary (and secondary muscles are usually short) or else the volsella is not part of the telopodite.

The morphological relationship between the gonopod and the volsella is emphasized by the detailed adult structure of the latter.

The separation of the volsella and gonostipes is anteriorly incomplete in some siricids, as in a species of *Sirex* (39, Fig. 15) and in both *Sirex juvencus* and *Xeris spectrum* (17, p. 228); the same phenomenon occurs in widely separated species of ichneumonids (Fig. 97), and is discussed later. While this is indicative of a similar morphological relationship between the inner claspers of these groups, yet, coupled with the primitive nature of the family Siricidae, it further suggests that the volsella originated from the gonoforceps.

The view that these two structures are primitively one is supported by the appearance of the upright ichneumonid basivolsella, in which (i) setae and alveoli are present facing the aedeagus, (ii) the muscles lie extrinsically and laterally and (iii) the curvature is towards the aedeagus. Together, these factors strongly suggest the rounded shape and musculature of the mesal portion of the gonopod. The setae are evidently primary, because they are small in comparison with the alveoli; furthermore, the setae and alveoli only occur postero-ventrally, as in *Ichneumon grotei* Cress. (Fig. 121), this being a position favored by the basivolsellar curvature for the survival of setae from the attritional effects of invagination, yet an unsuitable place for the development of tactile organs for use during coition. The curvature of the basivolsella is in itself insignificant, for the shape may possibly be due to the tension exerted by Boulangé's muscle Q (Fig. 8), although this muscle probably is only used during coition. Individually these three factors are not of much weight, yet, when correlated with each other and with the ontogenetic development of the volsella, they indicate that the volsella and gonoforceps in the Ichneumonidae are primitively one structure.

These facts, incidentally, serve also to show the falsity of the idea that the volsella may have originated directly from membrane, instead of from sclerotic structures; the form and musculature of the volsella are far too specialized to be associated with such an extensive change, one that is already so complete that there are no intermediate forms, even in the most primitive of the Hymenoptera.

Since the volsella and gonostipes are secondarily divided from each other, the gonopod consists of either three or four units, these being (i) the lateral half of the gonocardo, (ii) the gonostipes and volsella, (iii) the gonosquama and possibly (iv) the paramere of the aedeagus. Unfortunately, these units do not articulate sufficiently in any one plane to allow recognition of the coxopodite, subcoxa or coxa.

Neither can these parts be identified by their musculature. The longest muscles in the male hymenopterons are Boulangé's muscles I, J, M and N (Figs. 8, 10), all of these being attached to the base of the paramere and to the apex of either the gonostipes or the volsella. Consequently, if the para-

mere is held to be fundamentally separate from the gonopod, these muscles must be attached to the coxopodite and not to the telopodite, the gonostipes and volsella then being derived from the coxopodite. On the other hand, if the paramere is an appendage attached to the base of the gonopod, the gonostipes and volsella must still be derived from the coxopodite, else muscles are extending from the base of one appendage to the base of another. Crampton (24) has cited Snodgrass as considering this derivation to be the case.

However, this conclusion may seem to be invalidated by the gonocardo being constantly the first and only sclerite to be transversely differentiated from the gonopods. On this account, the two coxopodites may be regarded as being modified as the gonocardo. The morphological importance of the latter was emphasized by Pruthi (85), who suggested that it was probably homologous with the structures to be found at the base of the genitalia in the other main pterygote orders.

Assuming that both the gonopod and the thoracic limb are primitively divided into the coxopodite and telopodite, there would seem to be but one way of reconciling the ontogenetic and myological data. It can be put forward that the primary muscles of the gonopod have degenerated, owing to the extensive structural modifications that the gonopod has undergone. As pointed out by Abbott (2), there is no muscle capable of moving the ichneumonid claspers laterally; the genitalia then must be extended sideways by an increase in the blood pressure in this region. Blood pressure has already been noted as a factor in the functioning of the genitalia of the male honey bee (120), although in this insect there are no functional claspers and the aedeagus is mainly membranous. In the ichneumonids, too, the volsella is rotated from the vertical or resting position to the horizontal or copulatory attitude and, as there are no muscles capable of doing so, again an increase in the blood pressure in the neighborhood must be responsible. Should this modification be generally true of the Hymenoptera, then the arrangement of muscles is not a reliable source from which to draw fundamental, morphological conclusions.

The true nature of the hymenopterous gonopod, then, is not as yet definitely established. The median organ is an aedeagus, while the gonocardo *may* represent the fused coxopodites, the remainder then being derived from the telopodite. However, the stipes and volsella are primitively one. Until further evidence is found, it seems impossible to homologize with certainty the hymenopterous genitalia with the primitive limb.

B. The Ichneumonid Male Genitalia

1. *The Structure and Musculature*

The ichneumonid genitalia resemble those of the Chalastogastra, a closely related but less specialized group, in which however the genitalia vary greatly in form. The ichneumonids differ from the Chalastogastra in (i) the frequent formation of a syntergum or of a pair of syntergites, (ii) the almost invariable

absence of the parapenes, (iii) the absence of a distinct gonosquama in most species, (iv) the greater refinement or specialization of the volsella and its greater uniformity of shape and (v) the greater degree of sclerotization in the aedeagus. The ichneumonids are nearer to the parasitic sub-order Idiogastra, as represented by *Oryssus sayi*, although this species has a non-articulated gonosquama and its aedeagus is only weakly sclerotized.

The main homologies between the chalastogastrous genitalia and those of the ichneumonids are quite evident, if shape, position and articulation are sufficient criteria. This, however, does not apply to the inner claspers, and to show this relationship it is necessary to describe in detail the structure of the ichneumonid inner clasper. This appendage is assumed to be a volsella and the proof is shown subsequently.

The volsella of *Neotypus americanus* Cush. is typical of this structure as it occurs in the ichneumonids so that both the external (or mesal) and internal views of this organ in the vertical position have been shown (Figs. 119, 120). It should be noted that all drawings of this appendage show a large basal piece shaded by broken lines; these lines do not represent a completely desclerotized plate, but, instead, one that is extremely thin compared with the remainder of the volsella, almost resembling membrane in appearance. This plate is termed the *basivolsella* (Bv.) and is braced by the internal *volsellar strut* (V. Str.) which divides the basivolsella by passing from the antero-ventral margin to the postero-dorsal corner of the basivolsella. The anterior apex of the strut is developed into the *basivolsellar apodeme* (Bv. A), which is curved ventrally, laterally or dorsally. The postero-ventral margin of the basivolsella bears the strongly sclerotized *distivolsella* (Dv.) in which the apical margins are usually incurved and fused, forming a hood. The antero-dorsal margin of the distivolsella is prolonged into the *distivolsellar apodeme* (Dv. A.) to which is attached dorsally the gonolacinia (Gl.), which almost invariably is articulated.

The volsellae in most sawflies bear but little resemblance to the inner claspers of the ichneumonids, except that they have in common two distinct sclerites, the anterior *pièce complémentaire* of Boulangé (17) (which corresponds to the basivolsella and distivolsella) and the dorso-apical *pièce en trébuchet* (which is identified with the gonolacinia).

The position and articulation of the ichneumonid inner clasper is quite similar to that of the Chalastogastra. The same variation in articulation occurs in both, since the siricid volsella moves either feebly or not at all upon the gonostipes, although freely in the tenthredinids (17, p. 228); the former is true of a few species of ichneumonids, although the latter is far more common.

The volsellar strut is a constant feature of the ichneumonid inner clasper and is plainly identical in position with the diagonal nervure of Boulangé (17, pp. 62, 97), as shown in the siricids and in the tenthredinid, *Tenthredella mesomelas* L. (Fig. 117). It occurs also in the tenthredinid, *Dolerus similis*

Nort. (Fig. 118), in which the anterior end is developed into an apophysis, corresponding to the anterior apodeme of the ichneumonids.

The apex of the *pièce complémentaire* in both *T. mesomelas* (Fig. 117) and in *D. similis* (Fig. 118) shows a striking similarity to the ichneumonid distivolsella in having the apical hood and the antero-dorsal apodeme. Furthermore, this apodeme is present in *Oryssus sayi* (Fig. 113*) a member of the intermediate, parasitoidal Idiogastra.

Nevertheless, the diversification of form in the Chalastogastra suggests that there is a possibility that these similarities are due only to convergent evolution and thus are insignificant; this is supported by the view held by Boulangé that the inner clasper of the vespids is not homologous with the volsella of the Chalastogastra. On this account, the muscles of the chalastogastrous and ichneumonid genitalia (and the adjacent sclerites) have been compared.

The pimpline *Megarhyssa lunator* was selected for this work since its great size allowed the use of the binoculars instead of the microtome, the lack of appreciable amounts of melanin permitting the satisfactory transmission of light through the sclerites; the elongation of the abdomen and of the genitalia tends to isolate many of the muscles so that they can easily be recognized, but sometimes it also tends to change their axes. The muscles of *M. lunator* are compared with the morphological type of the Chalastogastra, as established by Boulangé (17), whose system of myological nomenclature is therefore followed (Figs. 7–11). Muscles D, E, F and L are not shown in the illustrations of *M. lunator* (Fig. 7) but their positions are described. A few of the muscles and their possible functions in *M. lunator* have already been briefly described by Abbott (2).

The abdomen of *M. lunator* is both narrow and elongate, the sterna being so strongly invaginated that the ventral margins of the terga touch in places. No muscles appear to join the tergum of one abdominal segment to the sternum of the immediately anterior or posterior segment (Fig. 5); the absence of this type of oblique muscle identifies the lateral parts of the syntergites as the genital tergites (Fig. 6), their fusion to the tenth tergites being incomplete posteriorly. The posterior boundary of the latter segment is indefinite, since the tenthredinid pygopods are borne upon the tenth tergum (124), although the ichneumonid pygopods are invariably separated from the tenth tergum by some membrane, this space being accentuated in *Megarhyssa* so that it resembles the eleventh tergum.

The syntergites are joined to the ninth sternum by a well developed muscle, Boulangé's *muscle en sangle.* The hypandrium is joined to the gonocardo by muscles A, B and C; the first two extend from the spiculum to the gonocondyle and to the antero-lateral margin of the gonocardo respectively, lying closely together, as in *Sirex juvencus.* Muscle C is weak but distinct in *M. lunator,* extending between the latero-posterior portion of the hypandrium

**This figure supports the tentative conclusions held by Crampton (22) in regard to the volsellar structures in the Oryssidae.*

and the gonocondyle. The ichneumonid gonocondyle resembles that of Orthandria in being either minute or absent; the development of the gonocondyle is associated with the lateral torsion of the genitalia in the Strophandria (22)*.

The gonocardo is joined to the basal margin of each gonostipes by muscles D, E, F and G; D and E cross each other ventrally, while F and G are dorsal. Muscle D is attached medianly to the gonocondyle and E similarly to the gonostipital arm. As *M. lunator* has long gonostipital arms, D and E lie dorsally to the gonocardo, E being short. Muscle F extends laterally from the antero-median portion of the gonocardo and is rudimentary, for this part of the gonocardo in *M. lunator* is membranous. Muscle G joins the antero-lateral margin of the gonocardo to the antero-median margin of the gonostipes, and its direction has been changed from being almost transverse to nearly longitudinal, owing to the sagittal elongation of the gonocardo; G has therefore assumed a part of the function of F.

Five muscles, H–L, join the gonostipes to the aedeagus. Muscle H is narrowly attached to the base of the paramere and broadly to the base of the gonostipital arm; it is well developed in *Megarhyssa;* its function is that of raising the apex of the aedeagus, which is balanced about the ergots by membrane and muscles. Muscle I, connecting the gonostipital arm to the ergot, is strong in *M. lunator* and serves to retract the aedeagus, sharing this function with the slender muscle K, which extends antero-dorsally from between the ergots to the gonostipes. Muscle J causes the exsertion of the aedeagus and connects the base of the paramere to the postero-dorsal part of the gonostipes; J is long and strong in *Megarhyssa* and in the Tenthredinids, although short in *Sirex*, *Bombus* and *Vespa* (17). The length of J in *M. lunator* may be due to the elongation of the gonostipes but then the same should be true of *Vespa*, unless only the apical part of the vespid gonostipital area has been developed posteriorly. Muscle L extends laterally from the ergot to the gonostipes and is both short and weak in *Megarhyssa*.

In *Megarhyssa* the gonosquama appears to be incompletely fused; muscle T is present, extending from the baso-lateral portion of the gonostipes to the gonosquama; the short gonosquamo-gonostipital muscle U and the intrinsic gonosquamal muscle V were not identified in *Megarhyssa*, perhaps because the gonosquama does not articulate in this species.

The volsella articulates only weakly in the siricids but strongly in the tenthredinids (17) so that the muscles of the ichneumonid volsella should resemble those of the latter group. Muscles O and P unite the volsellae to the gonostipites. Muscle O is absent in the Orthandria and in *M. lunator* but joins the base of the tenthredinid basivolsella to the apico-lateral part of the gonostipes. Muscle P, joining the antero-lateral margin of the gonostipes to the distivolsella, is present in all of these groups; it is well developed in *Megarhyssa* and is attached broadly at the base (this is not shown in Fig. 7).

**In the Strophandria or Tenthredinoidea the male genitalia rotate through 180° on the median axis before eclosion; this does not occur in the Orthandria (17, 22, 108).*

Two muscles, M and N, join the volsella to the aedeagus. The former extends from the apex of the gonolacinia to the extreme base of the paramere and is absent both in the Tenthredinidae and in *M. lunator*, although present in siricids. Muscle N joins the chalastogastrous gonolacinia basally to the base of the paramere. A moderately weak muscle is attached to the base of the gonolacinia in *Megarhyssa* but its basal attachment was not definitely established.

The volsella has three intrinsic muscles, Q, R and S, each attached to the base of the basivolsella and extending respectively to the apex of the distivolsella, the apex of the gonolacinia and the base of the gonolacinia. Muscle Q is present in the siricids and is large in *M. lunator;* in the tenthredinids it appears to be attached to the gonolacinia but Boulangé (17, p. 71) is not definite upon this point. In *M. lunator* the attachment is definitely at the basal apodeme of the distivolsella. Muscle R is absent in *Megarhyssa*, as there are no muscular fibres visible within the gonolacinia, while S seems to be reduced to some weak fibres.

The muscles of *M. lunator* disagree in detail with those of either the Orthandria or Strophandria alone, but when these are considered together, there can be no doubt that the gonocardo, gonostipes, gonosquama, basivolsella, distivolsella, gonolacinia and aedeagus are homologous in the three groups, the basi- and distivolsella together corresponding to Boulangé's *pièce complémentaire.*

2. *Function*

While much has been written upon courtship among the Hymenoptera, including some ichneumonids, only a few inadequate references have been made to the function of the genital parts of the male ichneumonid during coition or, indeed, even to the coital attitude of the male.

As early as 1799, De Geer (41) described the seizure of the female subgenital plate (*i.e.*, the eighth sternum) by the outer claspers of a male ichneumon, while Rohwer (100) noted that the inner claspers and aedeagus in the sawfly *Euura macgillrayi* Roh. were inserted into the genital cavity at the base of the subgenital plate. Neither Boulangé's own work nor his review of the literature adds anything further in regard to the functioning of the inner claspers and aedeagus.

The usual ichneumonid position of copulation seems to be that of *Pimpla instigator*, in which the male is dorsal to the female with his abdomen curved below the tip of the female's and somewhat to one side in order to avoid the ovipositor. In *Megarhyssa lunator* the male lies above the female with the apex of his abdomen below that of the female and the aedeagus is inserted from the anterior direction, since the female gonopore opens anteriorly (1, 2). In *Paniscus*, the male finally assumes a pendent position (130); this may be true of *Angitia fenestralis* Hlmgr. also, for specimens taken *in copula* were only loosely held together after being killed in alcohol and this may be due, not to chemical reactions upon the muscles, but to the adults having died

before the pendent attitude was reached. This variation in copulatory position suggests that there may be a correlation between the coital attitude and structure, as suggested by Abbott (2).

In this regard, it is interesting to note that in *Xiphidria* (Chalastogastra : Orthandria) the copulation is dorsal (100), there being no transverse rotation of the appendages in this group (17), although in at least some of the Strophandria, the males and females mate while facing in opposite directions (17, 55, 100).

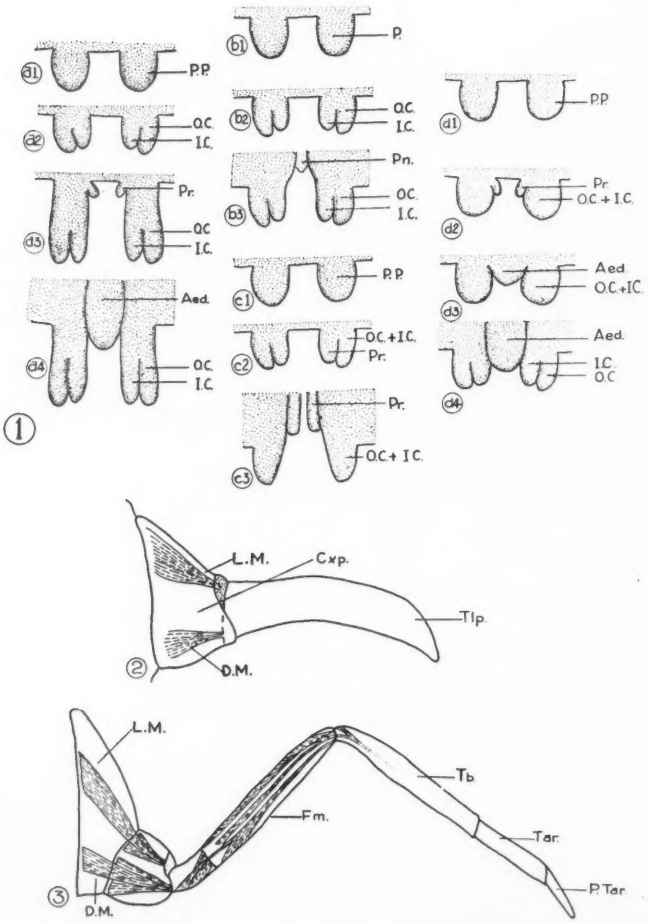
Specimens of the ichneumonid, *Angitia fenestralis*, taken *in copula*, were examined. In this species, the distal parts of the outer claspers grip the lateral portions of the subgenital plate of the female (Fig. 12), who no doubt extrudes her gonopore at the same time. The volsellae are rotated into the horizontal plane, owing to the muscular, ventro-posterior exsertion of the aedeagus and the increase in the blood pressure. The distivolsellae are bent upright through the contraction of muscle Q and the volsellar pincers are directed dorsally in respect to the male (but ventrally and slightly anteriorly with reference to the female). In this position, the pincers of the volsella are closed upon the intersegmental membrane of the female, owing to either the gonolacinial muscles or to the exserted aedeagus pulling the base of the gonolacinia posteriorly. The function of the volsellae is apparently to seize the conjunctival membrane, keeping it taut so that the aedeagus can be inserted into the female gonopore and be retained there. The aedeagus is exserted with its apex directed ventrally and, as it is strongly curved ventrally in this species, both its shape and its position at right angles to the main axis of the genitalia assist to retain the aedeagus in position during copulation. It should be noted that the function of the volsella was not definitely shown by dissections, because of the relaxations of the muscles but these conclusions were strongly indicated.

(To be concluded in next issue.)

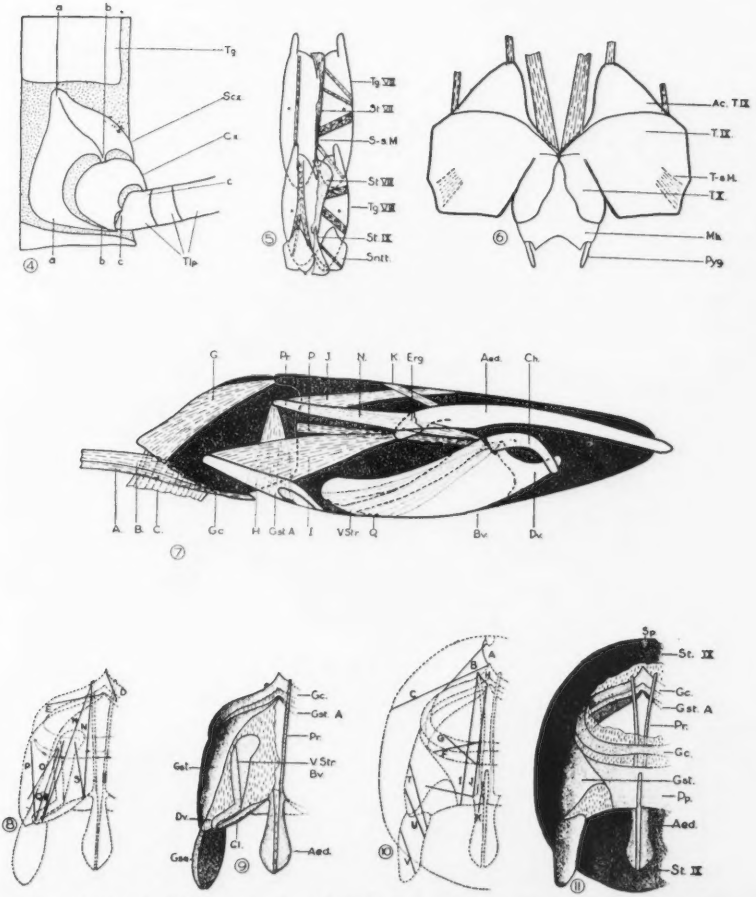
Abbreviations Used in the Illustrations

The abdominal segments are numbered with Roman numerals. The muscles of the male genitalia are lettered from A to V in accordance with the system of Boulangé (17) for the Chalastogastra.

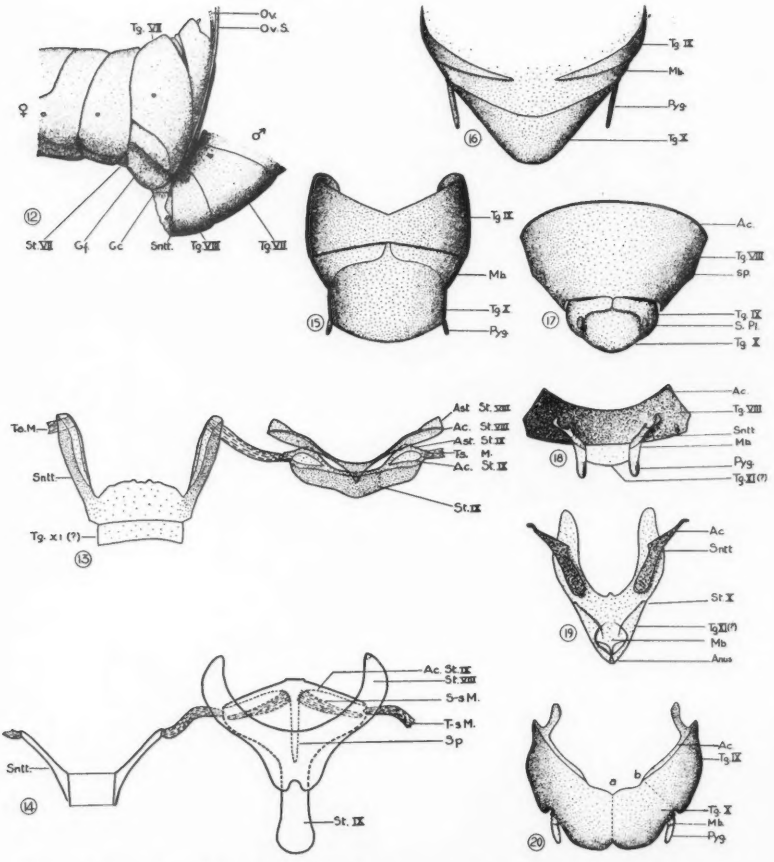
Ac.	antecosta	Ov.	ovipositor
Aed.	aedeagus	Ov. S.	ovipositor sheathe
Ast.	acrosternite	Pn.	penis
Bv.	basivolsella	P.P.	primary papilla
Bv. A.	basivolsellar apodeme	Pp.	parapenes
Cx.	coxa	Pr.	paramere
Cxp.	coxopodite	Ptar.	pretarsus
D.A.	dorsal area of the basivolsella	Pyg.	pygopod
D.M.	depressor muscle of telopodite	Scx.	subcoxa
Dv.	distivolsella	Snt.	syntergum
Ejac. D.	ejaculatory duct	Sntt.	syntergite
Erg.	ergot	Sp.	spiculum
Fm.	femur	S. Pl.	sensory plate
Gc.	gonocardo	Spt.	spatha
Gf.	gonoforceps	S-s.	intersternal
Gl.	gonolacinia	St.	sternum
Gsq.	gonosquama	Tar.	tarsus
Gst.	gonostipes	Tb.	tibia
Gst. A.	gonostipital arm	Tg.	tergum
Gtt.	gonotergite	Tlp.	telopodite
I.C.	inner clasper	T-s.	tergo-sternal
L.M.	levator muscle of the telopodite	T-t.	inter-tergal
M.	muscle	V.A.	ventral area of the basivolsella
Mb.	membrane	V.R.	ventral ridge of the aedeagus
O.C.	outer clasper	V. Str.	basivolsellar strut



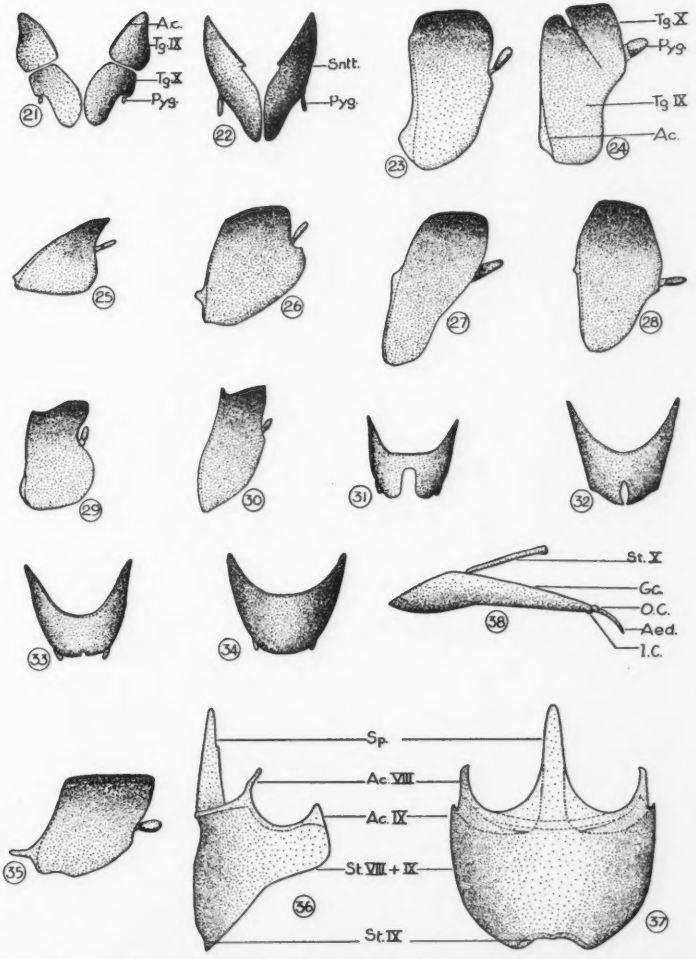
FIGS. 1–3. *Diagrams showing the developmental stages of the male genitalia in: (a) Sirex (according to Boulangé (17) and Vespa (Boulangé, after Zander (138). (b) Apis mellifica (according to Snodgrass (120), after Zander (138). (c) Apis mellifica (according to Michaelis (63). (d) Bombus (according to Boulangé, after Michaelis (63). 2. Diagram of the coxopodite and telopodite of a primitive limb, showing the levator and depressor muscles of the telopodite (after Snodgrass (124). 3. Diagram of a secondarily divided limb, showing the development of the secondary muscles (after Snodgrass (121, 124).*



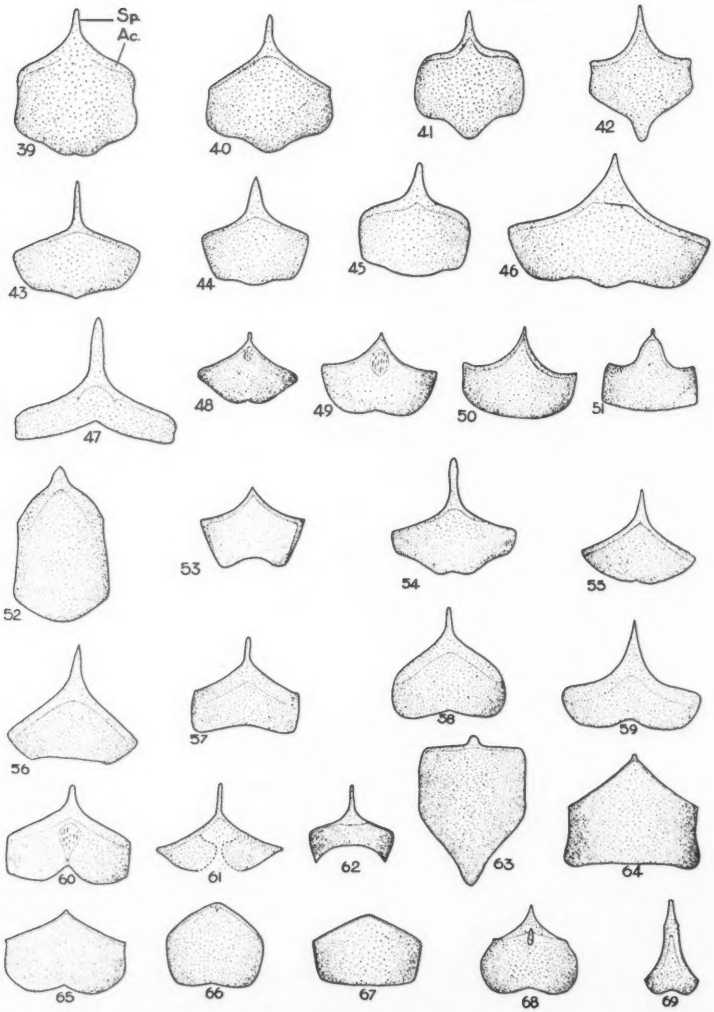
FIGS. 4–11. 4. *Diagram of a thoracic limb, illustrating the subcoxa, coxa and telopodite, together with their basal axes (a-a, b-b and c-c respectively) about which they move (modified after Snodgrass (122).* 5. *Megarhyssa lunator. Ventral view of abdominal segments VII-X, the terga spread horizontally and the left side of each sternum removed; tergal muscles placed to the left of the stippled sterna, the sternal and tergo-sternal muscles to the right.* 6. *Megarhyssa lunator. Dorsal view of the syntergites and their muscles, the syntergites placed in the horizonal plane.* 7. *Megarhyssa lunator. Inner aspect of the right half of the genital appendages, showing the muscles (exclusive of muscles D, E, F and L).* 8–11. *Diagrams of the genitalia and genital muscles in the Chalastogastra.* 8. *Ventral view of the genital muscles, the volsella placed in the horizontal plane; sclerites shown by broken lines and muscles by solid lines (redrawn after Boulangé, 1924).* 9. *Sclerites in Fig. 8 shown in relief; membranous areas shown by broken lines.* 10. *As in Fig. 8, but dorsal.* 11. *As in Fig. 9, but dorsal.*



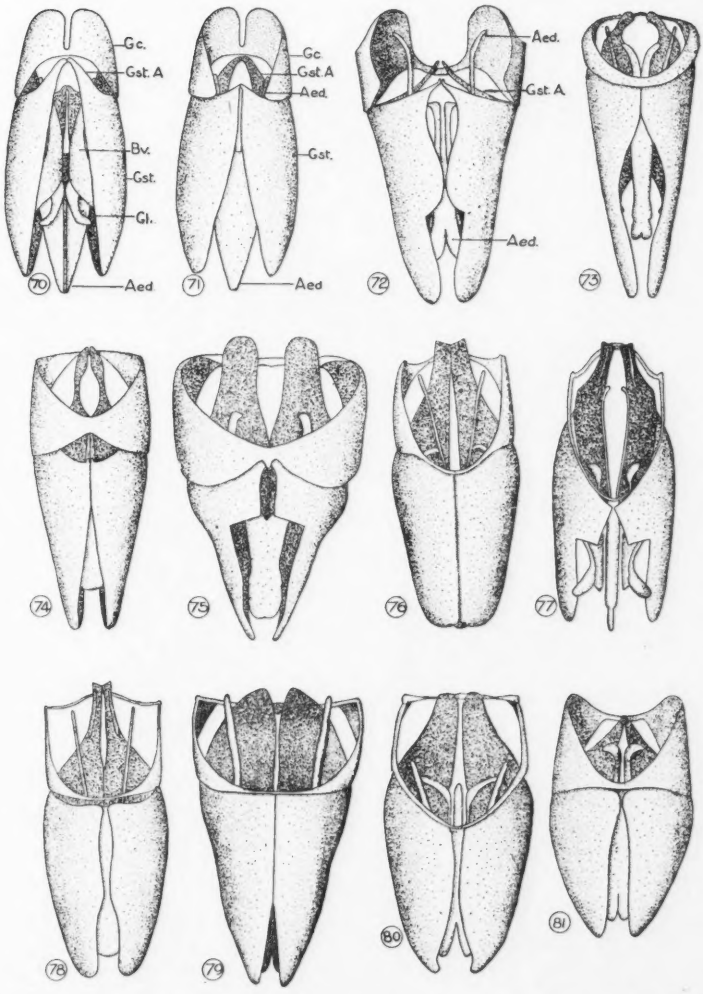
FIGS. 12–20. 12. *Angitia fenestralis. Lateral view of the terminal abdominal segments during copulation.* 13, 14. *External view of the syntergites, the eighth and ninth sterna, and the connecting muscles; stippled to show the degree of sclerotization.* 13. *Halictus lerouxii (Apoidea).* 14. *Andrena wilkella (Apoidea).* 15. *Cephus cinctus (Cephidae). Dorsal view of the terminal abdominal segments.* 16. *Pteronidea ribesii (Tenthredinidae). As above.* 17. *Brachymeria intermedia (Chalcididae). As above.* 18. *Lasius niger (Formicidae). As above.* 19. *Vespa maculata (Vespidae). As above.* 20. *Exeristes roborator (Ichneumonidae). Dorsal view of the syntergum, showing the line of median fission in the tenth tergum (a), and the line of inter-tergal fusion (b)*



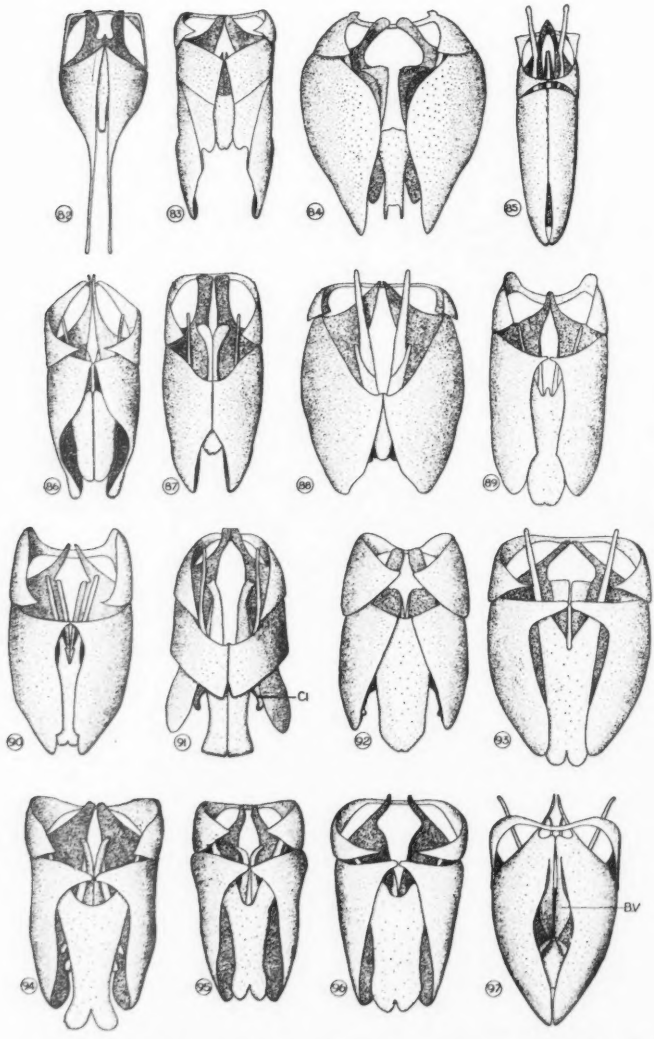
FIGS. 21–38. 21–35. *The ninth and tenth terga; the antecosta (Ac.) shown by a dotted line.* 21. *Pimpla (Iseropus) coelebs. Dorsal view of the tergites of the ninth and tenth terga.* 22. *Banchus falcatorius. Dorsal view of the syntergites.* 23. *Glypta fumiferanae. Lateral view of the ninth and tenth terga.* 24. *Glypta rufiscutellaris. As above.* 25. *Theronia fulvescens. As above.* 26. *Theronia melanocephala. As above.* 27. *Exetastes fascipennis. As above.* 28. *Exetastes matricus. As above.* 29. *Exenterus claripennis. As above.* 30. *Exenterus marginatorius. As above.* 31. *Cremastus flavo-orbitalis. Dorsal view.* 32. *Cremastus geminus. As above.* 33. *Cremastus minor. As above.* 34. *Cremastus incompletus. As above.* 35. *Itoplectis conquisitor. Lateral view.* 36. *Vespa maculata (Vespidae). Lateral view of hypandrium.* 37. *Vespa maculata (Vespidae). Ventral view of hypandrium.* 38. *Brachymeria intermedia (Chalcididae). Lateral view of the male genitalia and the tenth sternum.*



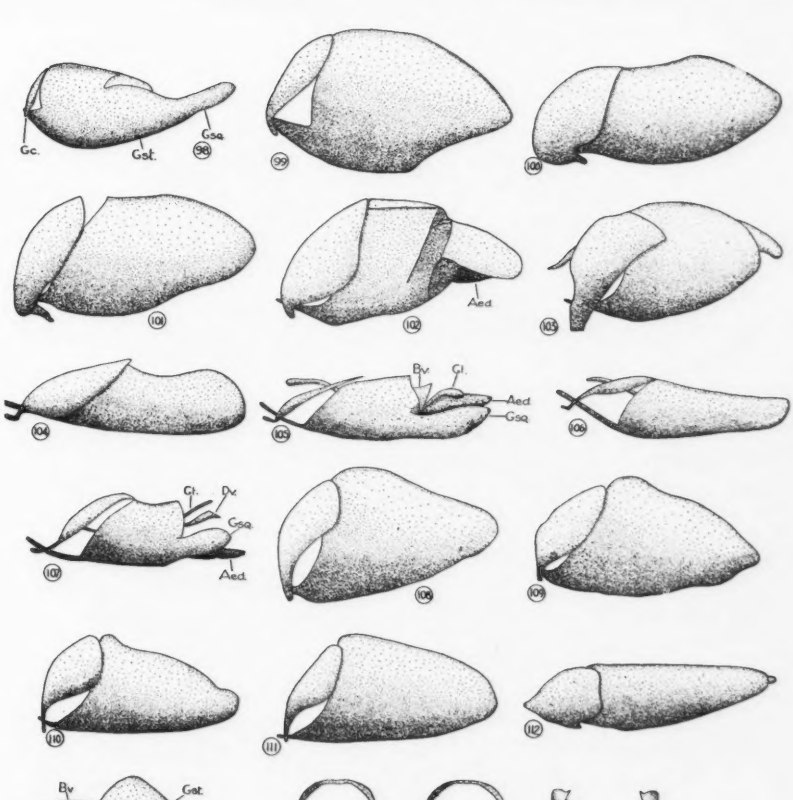
FIGS. 39–69. *Ventral view of the ninth sternum; membranous areas shown by broken lines.* 39. *Ichneumon variegatus.* 40. *Ichneumon animosus.* 41. *Ichneumon perscrutator.* 42. *Ichneumon grotei.* 43. *Phaeogenes gaspesensis.* 44. *Phaeogenes hariolus.* 45. *Hemiteles hemipterus.* 46. *Hemiteles subzonatus.* 47. *Hemiteles fulvipes.* 48. *Exetastes fascipennis.* 49. *Exetastes matricus.* 50. *Glypta rufiscutellaris.* 51. *Glypta fumiferanae.* 52. *Theronia fulvescens.* 53. *Theronia melanocephala.* 54. *Omorgus borealis.* 55. *Omorgus ensator.* 56. *Omorgus mutabilis.* 57. *Cremastus incompletus.* 58. *Cremastus flavo-orbitalis.* 59. *Cremastus minor.* 60. *Cremastus geminus.* 61. *Cremastus interruptor.* 62. *Pimpla coelebs.* 63. *Pimpla instigator.* 64. *Exenterus claripennis.* 65. *Exenterus canadensis.* 66. *Exenterus marginatorius.* 67. *Exenterus lepidus.* 68. *Tryphon incestus.* 69. *Megarhyssa lunator.*



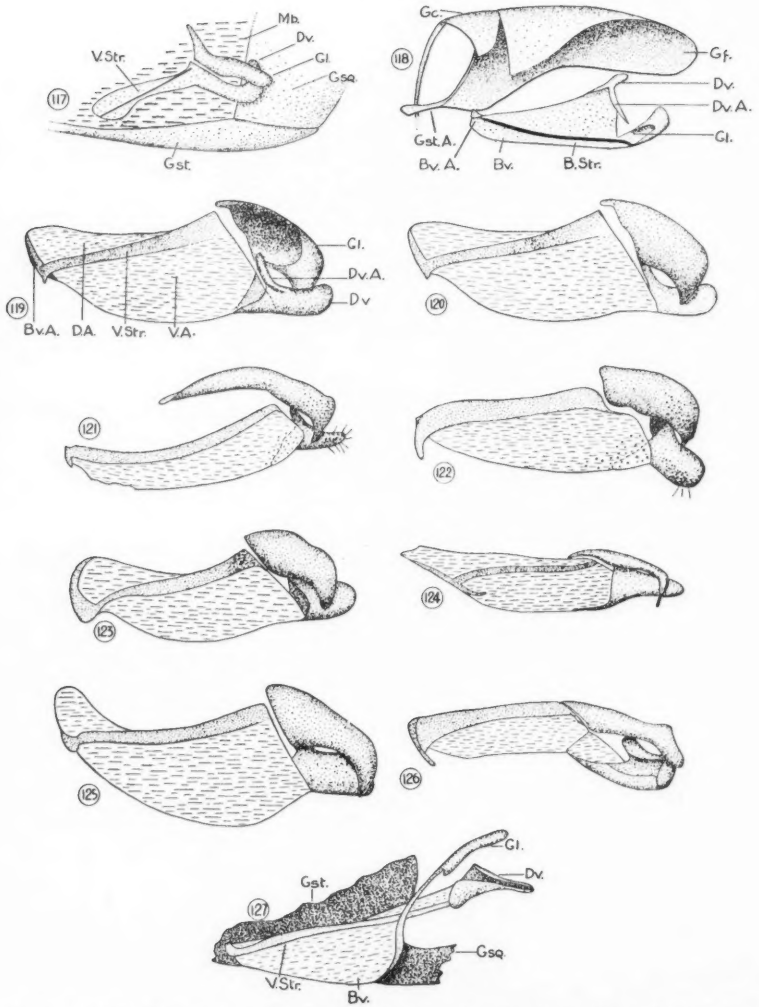
FIGS. 70–81. *The genitalia.* 70. *Megarhyssa lunator. Ventral view.* 71. *Megarhyssa lunator. Dorsal view.* 72. *Banchus falcatorius. As above.* 73. *Ophion obscurus. As above.* 74. *Agrypon flaveolatum. As above.* 75. *Exochilum circumflexum. As above.* 76. *Cremastus minor. As above.* 77. *Cremastus geminus. As above.* 78. *Cremastus flavo-orbitalis. As above.* 79. *Pristomerus vulnerator. As above.* 80. *Demophorus robustus. As above.* 81. *Orthopelma luteator. As above.*



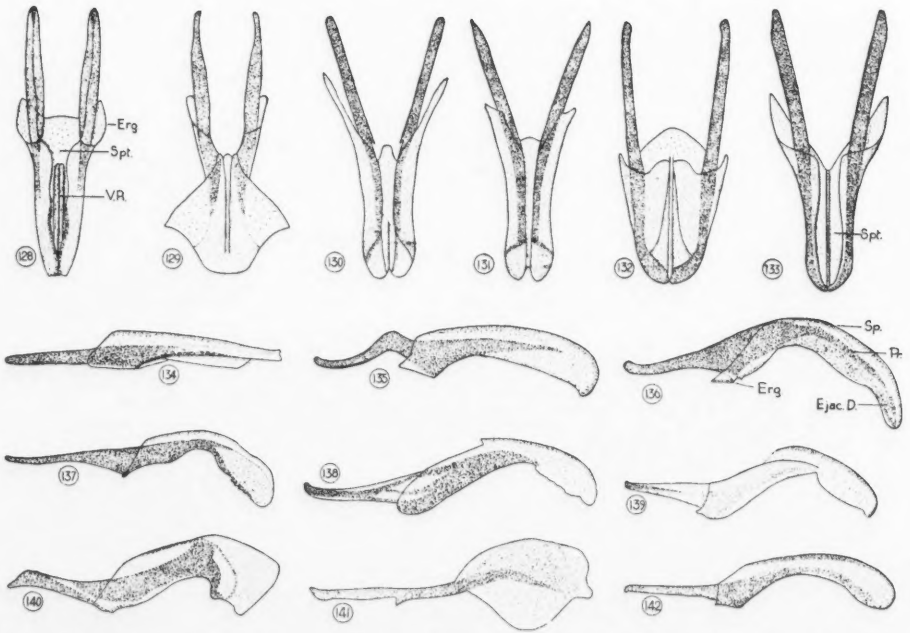
FIGS. 82–97. *The genitalia.* 82. *Mesochorus pectoralis. Dorsal view.* 83. *Hemiteles fulvipes. As above.* 84. *Hemiteles submarginatus. As above.* 85. *Enicospilus ramidulus. As above.* 86. *Apechthis ontario. As above.* 87. *Theronia fulvescens. As above.* 88. *Theronia melanocephala. As above.* 89. *Glypta rufiscutellaris. As above.* 90. *Glypta fumiferanae. As above.* 91. *Ephialtes tuberculatus. As above.* 92. *Ephialtes grapholithae. As above.* 93. *Exenterus canadensis. As above.* 94. *Exenterus claripennis. As above.* 95. *Exenterus lepidus. As above.* 96. *Exenterus marginatorius. As above.* 97. *Ichneumon longulus. Ventral view, showing the basal fusion of the volsella to the gonostipes.*



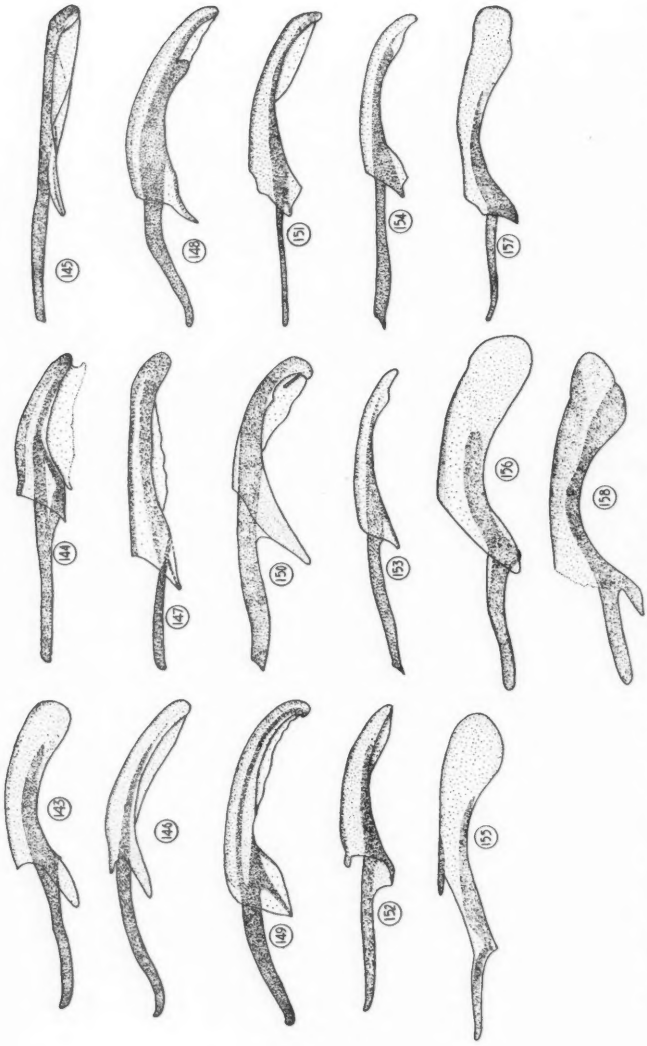
FIGS. 98–116. 98. *Hemiteles fulvipes. Lateral view of the genitalia.* 99. *Hemiteles submarginatus. As above.* 100. *Glypta rufiscutellaris. As above.* 101. *Glypta fumiferanae. As above.* 102. *Ephialtes tuberculatus. As above.* 103. *Ephialtes grapholithae. As above.* 104. *Cremastus interruptor. As above.* 105. *Cremastus geminus. As above.* 106. *Cremastus minor. As above.* 107. *Cremastus incompletus. As above.* 108. *Exenterus canadensis. As above.* 109. *Exenterus claripennis. As above.* 110. *Exenterus lepidus. As above.* 111. *Exenterus marginatorius. As above.* 112. *Megarhyssa lunator. As above.* 113. *Oryssus sayi (Oryssidae). As above, but the gonocardo absent.* 114. *Ichneumon longulus. Posterior view of gonocardo.* 115. *Ichneumon variegatus. As above.* 116. *Megarhyssa citraria. Dorsal view of gonocardo.*



FIGS. 117–127. *The volsella.* 117. *Tenthredella mesomelas (Tenthredinidae). Ventral view of the volsella in the horizontal plane and the adjacent sclerites (redrawn and shaded after Boulangé, (17).* 118. *Dolerus similis. Internal view of the widely separated volsella and gonoforceps.* 119. *Neotypus americanus. Lateral or internal view.* 120. *Neotypus americanus. Mesal or external view.* 121. *Ichneumon grotei. As above but showing portion of the basivolsella, torn from the gonostipes.* 122. *Ichneumon animosus. External view.* 123. *Hemiteles hemipterus. As above.* 124. *Enicospilus ramidulus. As above.* 125. *Cryptus annulatus. As above.* 126. *Cremastus minor. As above.* 127. *Cremastus incompletus. As above but showing the adjacent parts of the gonoforceps.*



FIGS. 128–142. *The aedeagus.* 128. *Megarhyssa lunator. Ventral view.* 129. *Agrypon flaveolatum. As above.* 130. *Glypta fumiferanae. As above.* 131. *Glypta rufiscutellaris. As above.* 132. *Theronia fulvescens. As above.* 133. *Theronia melanocephala. As above.* 134. *Megarhyssa lunator. Lateral view.* 135. *Ichneumon perscrutator. As above.* 136. *Ichneumon grotei. As above.* 137. *Ichneumon variegatus. As above.* 138. *Phaeogenes gaspesensis. As above.* 139. *Phaeogenes hariolus. As above.* 140. *Exochilum circumflexum. As above.* 141. *Agrypon flaveolatum. As above.* 142. *Glypta fumiferanae. As above.*



FIGS. 143–158. *Lateral views of the aedeagus.* 143. *Glypta rufiscutellaris.* 144. *Theronia fulvescens.* 145. *Theronia melanocephala.* 146. *Ephialtes grapholithae.* 147. *Ephialtes tuberculatus.* 148. *Pimpla brevicornis.* 149. *Pimpla coelebs.* 150. *Pimpla detrita.* 151. *Pimpla instigator.* 152. *Iioplectis conquisitor.* 153. *Iioplectis obesus.* 154. *Iioplectis pedalis.* 155. *Exenterus canadensis.* 156. *Exenterus claripennis.* 157. *Exenterus lepidus.* 158. *Exenterus marginatorius.*